American Academy of Allergy, Twelfth Annual Meeting, Chase–Park Plaza Hotels, St. Louis, Missouri, February 6, 7, 8, 1956

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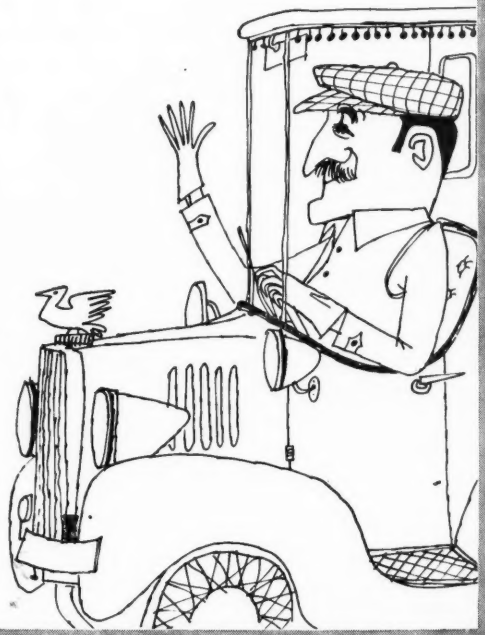

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The Journal of Allergy

VOL. 27 JANUARY, 1956 NO. 1

Original Articles

A CONTROLLED STUDY ON THE USE OF PARENTERAL AND ORAL ANTIHISTAMINES IN PREVENTING PENICILLIN REACTIONS

KENNETH P. MATHEWS, M.D., FAY M. HEMPHILL, PH.D., ROBERT G. LOVELL, M.D., WARREN E. FORSYTHE, M.D., SC.D., AND JOHN M. SHELDON, M.D.,
ANN ARBOR, MICH.

MUCH has been written about penicillin allergy. Even by 1948, Brown[1] found well over 300 articles to review on this subject, and numerous publications have appeared since then. A perusal of the early case reports reveals that more than fifty different types of alleged penicillin reactions have been described, although all these are not allergic in type. As to the incidence of reactions, statistics may be found indicating reaction rates varying from less than 1 per cent[2] to 53 per cent,[3] with all gradations between.

These comments are made not to compound the confusion but, rather, to emphasize how difficult it is to reach valid conclusions in this field. No doubt a major factor at the root of the difficulty is that there is no reliable, objective test for proving the diagnosis of all types of penicillin allergy. It is true that patch testing is a relatively reliable procedure in the occasional cases of penicillin contact dermatitis which are still encountered, and positive scratch or intracutaneous tests usually are obtained in the rare cases of anaphylactic type penicillin hypersensitivity. However, there has evolved a rather strong consensus, with which we are in agreement, that skin testing is not generally reliable in evaluating the vast majority of penicillin reactions. Thus, the diagnosis of penicillin allergy usually must be based on clinical judgment, and it is not surprising, therefore, that the statistics of various investigators are not entirely comparable.

In addition to various sources of error, there are a number of factors inherent in the data themselves which account in part for variations in the

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The Chlor-Trimeton used in this study was generously provided by the Schering Corporation through the courtesy of Dr. George Babcock, Jr.

Received for publication March 14, 1955.

reported incidence of penicillin reactions. There is some reason to believe that the type of penicillin preparation used, its route of administration, dose, and duration of treatment have some influence on the incidence of reactions. The patient's age and sex, the amount of previous penicillin therapy, a history of previous allergy or of mycotic infections, and a history of local reactions at the site of earlier penicillin injections also have been correlated with the incidence of penicillin allergy by various observers. However, with some exceptions (most notably the statistical study of Lepper and his associates[4]), many of these correlations are based on general clinical impressions without adequate documentation. A secondary objective of this study is to test the correlation of these factors with the incidence of penicillin reactions in a relatively large group of subjects.

Interest in penicillin allergy has been heightened in the past few years by many reports of severe or fatal reactions of an anaphylactic type.[5-7] There is reason to believe that these alarming reactions are increasing in frequency and will constitute an even more serious problem in the future. Thus, the problem of preventing penicillin reactions is assuming more and more importance. There is general agreement that this valuable drug should not be used indiscriminately. The topical use of penicillin on skin or mucous membranes should be discouraged, since it is attended by a high incidence of sensitization. Before prescribing penicillin, one always should ask the patient whether he had a reaction from any previous course of the drug, and a different antibiotic generally should be used (as is usually possible) when such a history is elicited. Preliminary skin testing may be of value when there is any possibility that the patient may be anaphylactically sensitized. Injections should be given in extremities, and a tourniquet and appropriate medications should be at hand for treatment of any possible severe reaction.

In spite of these precautions, there continue to be substantial numbers of unpredictable penicillin reactions. Further attempts at reducing the incidence of penicillin reactions have taken several forms, including the use of oral penicillin, penicillins O and BT, l-ephenamine penicillin (Compenamine), antihistamines mixed with penicillin, and procaine amide used in a similar manner. Reports of success with all these measures should be reviewed critically. We feel that valid conclusions can be reached only if the following requirements are satisfied:

1. Each study must contain its own control group. Comparison with reaction rates quoted in the literature is inadequate in view of the tremendous variations in the latter.

2. Study and control groups should be similar in respect to the type of patients observed and general program of penicillin therapy. The latter information should be included in the report in order to facilitate comparison with other studies. For example, it is not valid to compare with adult reaction rates the incidence of reactions to supposedly hypoallergic penicillins in groups composed largely of children, since there is good reason to believe that children normally

experience much less penicillin allergy than adults. Nevertheless, such comparisons have frequently been made in the literature.

3. Control and study observations should be made concomitantly and possible reactions evaluated without knowledge as to the group to which individual patients belong. This is necessary because the diagnosis of penicillin allergy usually rests on clinical judgment, rather than on an objective test.

4. Patients should be followed for reactions for several weeks after completion of penicillin therapy, since reactions frequently are delayed in onset. Specific inquiry must be made about delayed reactions, as our experience indicates that patients may neglect to report delayed reactions voluntarily when they are mild in degree.

5. Large numbers of patients must be observed to achieve statistically significant results. The level of confidence should be stated in the report.

6. Failure of previously penicillin-sensitive persons to react to a subsequent course of the drug does not prove a hypoallergic effect, since penicillin allergy often is spontaneously lost over a variable period of time.

7. Absence of anaphylactic type reactions in series of a few thousand cases does not prove a hypoallergic effect, because at present these severe reactions still occur relatively infrequently with all forms of penicillin.

Among the methods suggested for reducing the incidence of penicillin reactions, the use of antihistamines for this purpose offers several advantages: these drugs are readily available, there is much information regarding their toxicity, side effects, and pharmacologic properties, and they do not appear to affect the therapeutic efficacy of the penicillin.[8] There also is no doubt that these drugs sometimes are effective in treatment of penicillin reactions, particularly those of an urticarial type. Thus, it is logical that the prophylactic administration of antihistamines might reduce the incidence of such reactions, and a number of reports[9-19] have appeared in the literature asserting that this is the case. In fact, the publications appearing thus far have indicated a virtual elimination of all allergic reactions to penicillin by means of antihistamines. Furthermore, these amazing results have been achieved simply by using solutions of antihistamines as diluents for the penicillin solution before injection, a procedure which assures the patient's receiving the drug but with no added inconvenience. In addition, local reactions and discomfort at the site of injection are said to be reduced markedly by this procedure, and there seem to be less side effects from the antihistamine when it is given by injection.

The practical importance of this work is obvious. On a priori grounds, however, the previously reported results seem rather surprising. Although it is logical that antihistamines might suppress some allergic reactions during

the period of their pharmacologic effect, this period is generally thought of in terms of a number of hours following the administration of these drugs, and it is difficult to understand how they would inhibit delayed reactions which may not begin for many days. There is no convincing evidence that antihistamines inhibit antibody formation in general, and Dreisback[20] did not observe any inhibition in the development of experimental penicillin hypersensitivity in rabbits by the administration of antihistamines. Furthermore, although antihistamines might be expected to suppress urticarial reactions, several of the cutaneous manifestations of penicillin allergy are not known to be associated with histamine release, and yet the previously cited reports have indicated a virtual elimination of all types of penicillin reactions. In view of these considerations and the fact that most of the studies reporting favorably on the use of antihistamines do not satisfy the previously discussed requirements for definitive work in this field, it was thought worth while to report our controlled study on this subject.

PROCEDURE

This study was carried out during the twenty-two-month period ending in August, 1954, at the University of Michigan Student Health Service with the cooperation of the staff there. Every patient for whom penicillin was prescribed by any department of the Health Service was sent to the allergy clinic to receive the drug. Before this was given, he was requested to cooperate in the study by taking tablets designed to prevent penicillin reactions. He also was asked to fill out a form, giving pertinent information about his age, sex, number of previous courses of penicillin, routes of previous penicillin therapy, time interval since last penicillin, past penicillin reactions, other drug allergies, history of cutaneous fungus infections, personal history of the common allergic diseases, and family history of allergy. The procedure in hospitalized cases also was supervised by the allergy clinic. Patients retained a second form which was to be returned to the allergy clinic *no sooner* than three weeks after their last dose of penicillin. This form indicated the route, frequency of administration, and duration of penicillin therapy, the regularity with which the "preventive tablets" were taken, the nature and severity of any side effects noted from the "preventive tablets," and detailed information about any possible penicillin reactions. The latter included a description of the reaction, time of onset, duration, severity, and associated local reaction at the site of injection. In addition, patients were urged to return to the allergy clinic at the first sign of itching, rashes, or other possible manifestations of a penicillin reaction, and the information on the questionnaire concerning the reaction was amended as indicated by the evaluating physician.

The major portion of the study was designed so that patients received, in strict rotation, fortified aqueous crystalline procaine penicillin G (S-R penicillin) diluted either with physiologic saline solution or with a solution containing 10 mg. of chlorprophenpyridamine (Chlor-Trimeton). Patients in each of these groups also were given, in rotation, either 8 mg. repeat-action Chlor-Trimeton tablets or similar placebo tablets to be taken three times daily

for the duration of penicillin therapy and for ten days thereafter. It was thought that prolonged oral administration of antihistamines might offer maximal opportunity for these drugs to show a beneficial effect, although it was realized from the outset that many of the patients would fail to take pills regularly for such a long period. Since there is some evidence that salicylates can inhibit the development of certain manifestations of serum sickness,[21, 22] which delayed penicillin reactions sometimes resemble, in the early phases of this experiment there also was included a group of patients who received aspirin. When it became apparent that this drug was having no effect in the dosage used (2.65 Gm. per day), it was abandoned and the data were included with the placebo group. Our data do not exclude the possibility that much larger doses of salicylates might have some beneficial effect, but it was not possible for us to attempt further studies of this type under the conditions of our experiment.

All possible reactions were evaluated by a single investigator without knowledge as to which type of medications the patient had received. Criteria for the diagnosis of a penicillin reaction were: (1) clinical manifestations of a type known to be related to penicillin allergy; (2) a satisfactory time relationship between these manifestations and penicillin administration; and (3) the absence of other apparent cause for the observed reaction. Only those cases which seemed to represent definite penicillin reactions were included in the major portion of the statistical analysis. A separate record and analysis was made of the more questionable cases in which penicillin allergy could not be excluded but did not seem to be definite. The pre- and posttreatment questionnaires submitted by the patients were reviewed and amended as indicated by the investigators. They were then coded, punched on cards, and tabulated with the aid of IBM machines, a procedure which greatly facilitated handling the more than 100,000 items of data which accumulated during the course of the study.

As had been anticipated from the beginning, there were two problems which could not be entirely surmounted in carrying out this experiment. One was that although the patients were strongly urged to return *at the time* they experienced any possible symptoms or signs of penicillin reactions, they sometimes failed to do so, especially when their symptoms were mild. In such instances, they were contacted and personally interviewed by the evaluating investigator, such possible reactions usually being relegated to the "questionable reaction" group unless the patient's description was clearly and completely characteristic of a penicillin reaction. Although this procedure was not ideal, it would be very hard, indeed, to achieve conditions under which very large numbers of patients could be followed more closely for some period of time following completion of penicillin therapy.

The other major problem was failure to take the "preventive tablets" as prescribed. Forty-five per cent of the patients in the entire study failed to take the tablets with reasonable regularity, in spite of strong encouragement

to do so. This would limit considerably the practicality of this form of therapy, although it is possible that under other conditions (such as if the patients had paid for the tablets) cooperation might have been better. Side effects of the antihistamines were not a major factor, since the record of pill taking was the same in the placebo pill group. Among those who had reactions to penicillin, pill taking was considered adequate if it had been reasonably regular for the prescribed period or up until the start of the reaction. Under these conditions, 11 per cent of penicillin reactors were considered to have taken their pills inadequately.

RESULTS

Table I shows the general characteristics of the group studied. The preponderance of male patients reflects the higher male student enrollment. As would be expected in a university student group, only two persons were under 17 years of age and thirty-seven were over 40 years of age. It is interesting that only 20 per cent of the students had never received penicillin before, whereas almost 40 per cent had received three or more previous courses. Very appreciable numbers had received the drug previously in dosage forms other than by injection.

TABLE I. GENERAL CHARACTERISTICS OF THE GROUP STUDIED (2,299 COURSES OF PENICILLIN)

Sex:	
Males	61.7 per cent
Females	38.3 per cent
Age:	
Median	21 years
Under 17 years	2
Over 40 years	34
Previous Penicillin Therapy:	
None	20 per cent
One course	24 per cent
Two courses	18 per cent
Three or more courses	38 per cent
Route of Previous Therapy:	
Injection	1,631
Oral tablets	426
Troches	413
Ointment	99
Other	44

Table II shows that of the 2,299 patients who entered the study, an adequate follow-up was achieved in all but 100, this better than 95 per cent follow-up being accomplished with the aid of hundreds of reminder postcards and telephone calls. Because of the danger of severe anaphylactic-like reactions to penicillin, we did not encourage the employment in this study of patients known to have reacted previously. The small group falling in this category is considered separately in the statistical analysis of our data. Likewise, except where otherwise specified, the major portion of our statistical analysis is confined to the 1,922 courses of penicillin given only by injection.

Table III shows the comparability of the four treatment groups. Group I, as indicated, is a control group given penicillin diluted with saline and oral

TABLE II

Total Courses of Penicillin:	2,299
Unable to follow	100
Followed for 3 weeks or more	2,199
Persons having reactions to previous courses of penicillin	47
	2,152
Route of Penicillin Administration:	
Injection only	1,922
Tablets only	106
Troches only	81
Ointment	1
Injection and tablets	28
Injection and troches	13
Not recorded	1

placebo tablets. Group II received saline penicillin and oral Chlor-Trimeton, Group III received Chlor-Trimeton penicillin and an oral placebo, and Group IV received Chlor-Trimeton penicillin and Chlor-Trimeton orally. A comparison of these data shows no very major differences among any of the groups in respect to the factors listed: age, sex, previous penicillin therapy, history of other drug allergies, history of cutaneous fungus infection, history of allergic diseases, or duration or dosage of penicillin treatment. Incidentally, one may be surprised at the very high percentage of patients in all groups giving a history of hay fever or asthma. This aspect of our data will be the subject of a further report.

TABLE III. COMPARABILITY OF TREATMENT GROUPS

	GROUP I SALINE PENICILLIN ORAL PLACEBO	GROUP II SALINE PENICILLIN ORAL CHLOR-TRIMETON	GROUP III CHLOR-TRIMETON PENICILLIN ORAL PLACEBO	GROUP IV CHLOR-TRIMETON PENICILLIN ORAL CHLOR-TRIMETON
Total courses of penicillin	501	443	478	491
Median age (years)	21	21	20	20
Sex:				
Male	66%	60%	59%	61%
Female	34%	40%	41%	39%
Previous penicillin therapy:				
None	25%	20%	19%	20%
Three or more courses	32%	40%	38%	42%
History of other drug allergies	7.0%	8.1%	7.3%	9.7%
History of cutaneous fungus infection	67%	59%	63%	60%
History of allergy:				
Allergic rhinitis	24%	21%	20%	25%
Asthma	8%	6%	8%	9%
Urticaria	16%	16%	17%	13%
Average days of treatment	2.8	2.7	2.8	2.8
Average number of injections per day	1.06	1.08	1.04	1.08

Fig. 1 shows the gross results of the study. There were sixty-four reactions to injected penicillin, an incidence of 3.3 per cent, distributed among the four groups as indicated. It is apparent that there is nothing approaching virtual elimination of penicillin reactions among the antihistamine groups. The statistical analysis of this and the following data was done by the Chi-

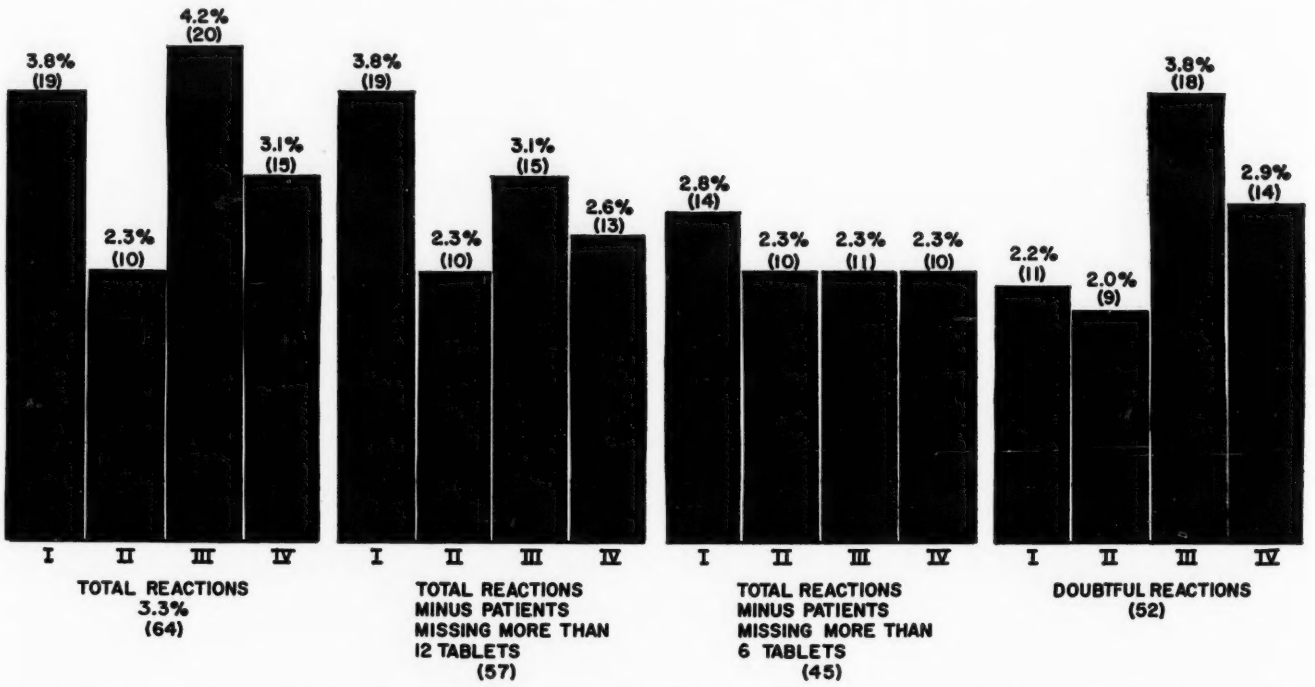

Fig. 1.—Group I was the control group given penicillin diluted with saline and an oral placebo; Group II received saline pencillin and oral Chlor-Trimeton; Group III received Chlor-Trimetcn penicillin and an oral placebo; Group IV received Chlor-Trimeton penicillin and oral Chlor-Trimeton.

square test (with Yates' correction), using a 5 per cent level of significance. The somewhat lower reaction rate among those receiving oral antihistamines in Groups II and IV is not statistically significant with this number of cases. The next two figures are presented to show the reaction rates after deleting those cases where the reacting patients did not take their pills regularly, but it is apparent that even less difference is observable between various groups after making this correction. Most of the subsequent statistical analysis is based on only the fifty-seven cases where the patients are known to have taken their pills with at least moderate regularity. As mentioned previously, a separate record was kept of questionable or doubtful cases where penicillin allergy was uncertain but could not be excluded. The last figure on Fig. 1 shows that inclusion of these cases in the main body of data would not have altered the results.

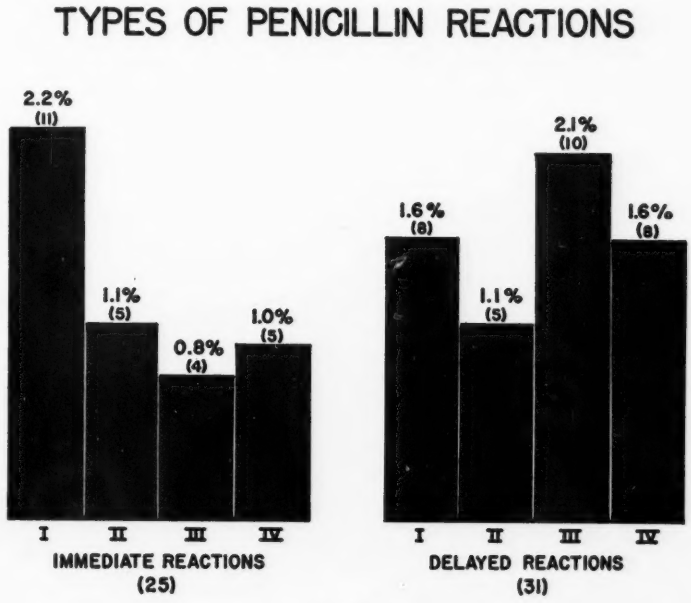

Fig. 2.

Fig. 2 indicates a subdivision of penicillin reactions into "immediate" ones coming on within forty-eight hours of starting penicillin therapy and delayed reactions beginning at any time thereafter. There is a statistically significant reduction in the incidence of immediate reactions among the three antihistamine-treated groups as compared with the control group. There is no significant difference in the incidence of delayed reactions. These results are in agreement with the theoretical expectations regarding the results of antihistamine administration.

The duration of the reactions which occurred was no longer in the control group than in the antihistamine groups, as indicated in Fig. 3. These data

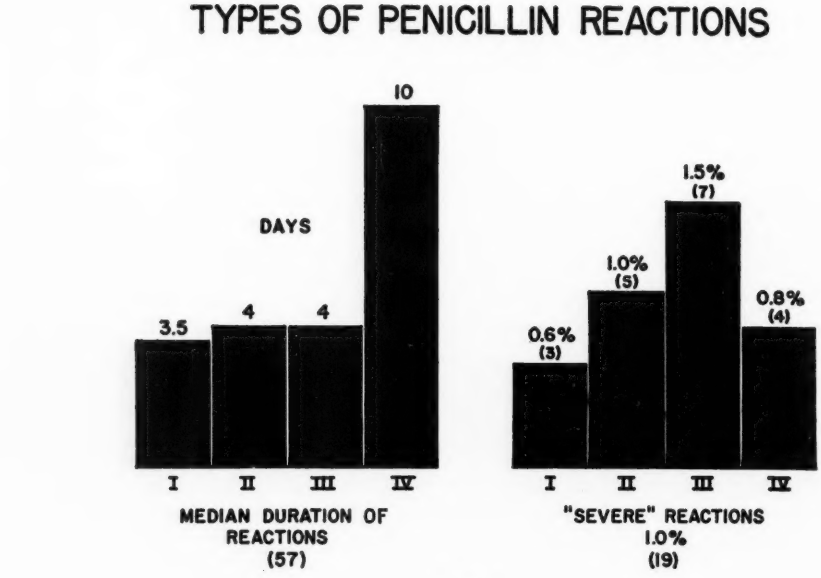
TYPES OF PENICILLIN REACTIONS
DAYS
3.5
4
4
10
I
II
III
IV
MEDIAN DURATION OF REACTIONS
(57)
0.6%
(3)
1.0%
(5)
1.5%
(7)
0.8%
(4)
I
II
III
IV
"SEVERE" REACTIONS
1.0%
(19)

Fig. 3.

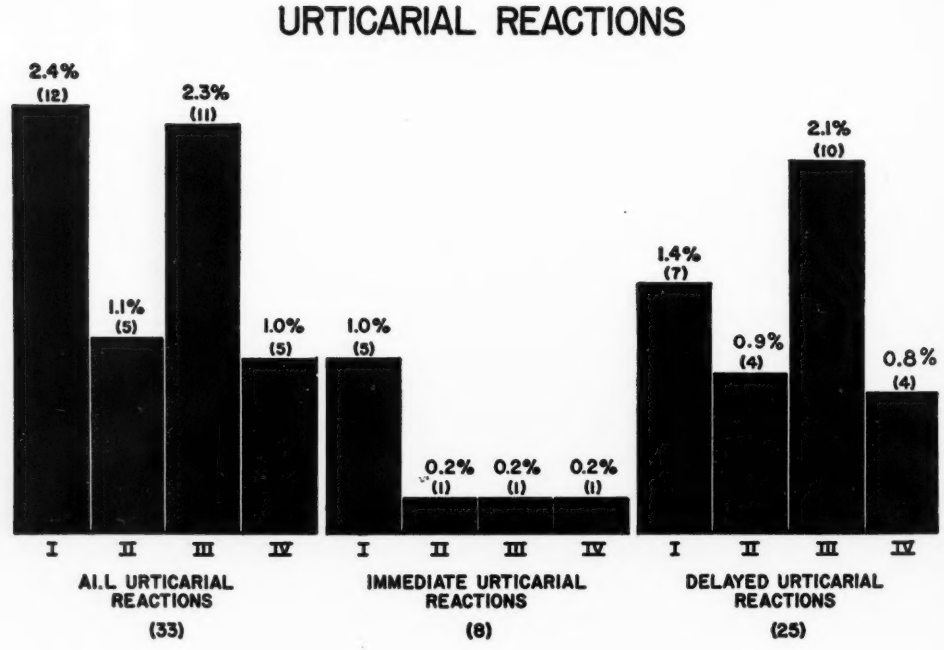
URTICARIAL REACTIONS
2.4%
(12)
1.1%
(5)
2.3%
(11)
1.0%
(5)
I
II
III
IV
ALL URTICARIAL REACTIONS
(33)
1.0%
(5)
0.2%
(1)
0.2%
(1)
0.2%
(1)
IMMEDIATE URTICARIAL REACTIONS
(8)
1.4%
(7)
0.9%
(4)
2.1%
(10)
0.8%
(4)
DELAYED URTICARIAL REACTIONS
(25)

Fig. 4.

are influenced by other treatment which was initiated once a penicillin reaction had definitely been diagnosed. The other figure in Fig. 3 is an important one. It must be admitted that many of the penicillin reactions encountered in this study were rather trivial, and antihistamine prophylaxis would be of value if it simply eliminated or modified the more severe reactions. This definitely was not the case. Even in Group IV, there were patients who required hormone therapy or hospitalization in the Health Service infirmary for relatively severe penicillin reactions. Seventeen of these nineteen reactions were of the delayed type. No anaphylactic type reactions were encountered in either the control or antihistamine-treated groups in this particular study.

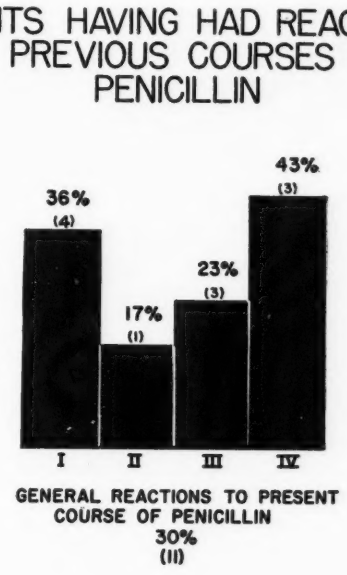

Fig. 5.

The incidence of urticarial reactions is specifically analyzed in Fig. 4, since it is in regard to this type of reaction that antihistamines would be expected to be most effective. There is a statistically significant higher incidence of urticaria in Groups I and III, not receiving prophylactic oral antihistamines, than in Groups II and IV which did receive these drugs orally. The other two figures in Fig. 4 show that this difference is accounted for to a considerable extent by a decreased incidence of immediate urticarial reactions. The difference in delayed urticarial reaction rates is not significant. These results all are in agreement with the previously discussed *a priori* expectations.

Fig. 5 shows the results in patients who gave what seemed to be a bona fide history of a previous allergic reaction to penicillin. The number of patients involved here is too small to enable one to draw any conclusions about differences in reaction rates among the different groups, which were of quite unequal size. The two points of importance are: (1) substantial num-

bers of these patients had reactions in spite of receiving oral or parenteral antihistamines and (2) 64 per cent of patients in the control group did not have reactions, again emphasizing the point that penicillin allergy may be lost spontaneously.

There were inadequate numbers of patients receiving penicillin by routes other than or in addition to injection to justify a statistical analysis of these groups. It is of interest, however, that what data are available do show a trend toward a relatively low reaction rate among those receiving oral penicillin and a high rate among those using troches, as shown in Fig. 6. This is in agreement with the literature. The one reaction from oral penicillin was rather trivial.

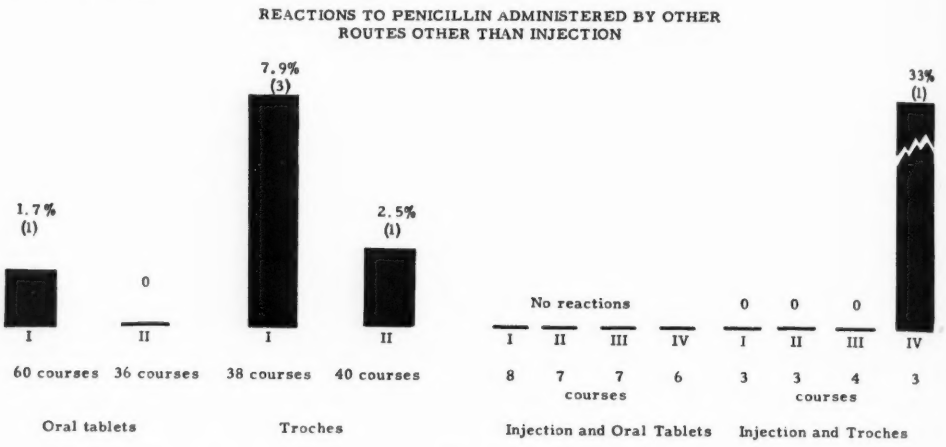

Fig. 6.

The types of reactions observed among those receiving penicillin by injection during this study are summarized in Table IV. Since quite a few patients exhibited more than one manifestation of penicillin allergy, the number of reactions listed here exceeds the number of patients who experienced them.

A secondary objective of this study has been to analyze the data for factors predisposing to penicillin reactions. Table V shows the incidence of various factors among those who had reactions and those who did not. There is no statistically significant sex difference. In regard to age, there was a trend toward more frequent reactions in the older age group, although this was not statistically significant. No appreciable differences were noted in the incidence of other drug allergies or cutaneous fungus infections between the two groups, although the accuracy of obtaining information about the latter by questionnaire might well be challenged. There was a slight trend toward a higher incidence of allergic rhinitis, asthma, and hives among the reactors than among the nonreactors, but this difference was not significant, and the trend regarding family history of allergy was in the opposite direction. These

TABLE IV. TYPES OF REACTIONS OBSERVED AMONG PATIENTS RECEIVING PENICILLIN BY INJECTION

TYPE OF REACTION	"IMMEDIATE" REACTIONS	DELAYED REACTIONS	TOTAL REACTIONS
Urticaria and/or angio-edema	8	26	34
Papulovesicular eruptions	7	2	9
Erythematous eruptions	5	3	8
Serum sickness syndrome	1	8	9
Other rashes	5	3	8
Others	1	1	2
Totals	27	43	70

observations are in agreement with those of many others that the lack of an atopic background is no assurance whatever that a patient will not have an allergic reaction to penicillin.

Table VI shows that there were no significant differences between the reactors and nonreactors in respect to duration of penicillin treatment or dosage. It should be emphasized, however, that almost all patients in this study received relatively small amounts of penicillin, and therefore, in certain

TABLE V. ANALYSIS OF FACTORS PREDISPOSING TO REACTIONS FOLLOWING PENICILLIN INJECTIONS

	REACTORS	NONREACTORS
Total courses of penicillin	64	1797
Sex:		
Male	55%	62%
Female	45%	38%
Age:		
Median	20 years	20 years
Average	21.9 years	21.3 years
Over 30 years	10.9%	5.4%
Under 19 years	20.3%	19.3%
History of other drug allergies	7.8%	7.7%
History of cutaneous fungus infection	56%	63%
History of allergy:		
Allergic rhinitis	29.5%	22.2%
Asthma	9.5%	7.7%
Urticaria	17.5%	15.1%
History of familial allergy:		
Allergic rhinitis	33.3%	25.8%
Asthma	12.7%	18.7%
Urticaria	11.1%	14.6%

respects, these data may not be applicable to other groups[4] receiving much larger quantities of the drug (for example, patients with subacute bacterial endocarditis). Although the average length of time since the last course of penicillin was very similar in the reactor and nonreactor groups as a whole, further analysis of the data shows that those persons who experienced delayed penicillin reactions significantly more often had not received penicillin within one year than either "immediate" reactors or nonreactors. It was rather surprising to find that, in this series of cases, the penicillin reactors had not received any more courses of penicillin therapy in the past than nonreactors. Previous use of penicillin ointment was noted more than twice as often in the reactor as in the nonreactor groups, although the number of cases involved

TABLE VI

	REACTORS		NONREACTORS
Average duration of therapy	2.88 days		2.77 days
Injections per day:			
One	92.2%		92.9%
Two or more	7.8%		7.2%
Time since last penicillin therapy:			
Average	14.0 months		14.3 months
	"Immediate"	Delayed	
Less than 1 year	67%	33%	56%
Over 1 year	33%	67%	44%
Number of previous courses of penicillin:			
None	29%		21%
One or two	45%		40%
Three or more	25%		39%
Route of previous penicillin:			
Injection	84%		93%
Tablet	20%		23%
Troche	22%		21%
Ointment	13%		6%
Other	4%		3%
Local reactions:			
None	50%		73%
Moderate or severe	22%		6%

was too small to make this difference statistically significant. Last, a significantly greater percentage of the penicillin reactors complained of moderate or severe local reactions at the injection sites than did those without general reactions.

TABLE VII. COMPARATIVE SEVERITY AND DURATION OF "IMMEDIATE" AND DELAYED REACTIONS

		"SEVERE"		LESS SEVERE
"Immediate" reactions	(25)	12%		88%
Delayed reactions	(36)	53%		47%
		1 or 2 Days' Duration	*3 to 7 Days' Duration*	*Over 7 Days' Duration*
"Immediate" reactions	(25)	40%	40%	20%
Delayed reactions	(35)	17%	46%	37%

Table VII shows that delayed reactions are likely to be more severe than "immediate" reactions by a statistically significant difference. An important exception to this generalization is the rare anaphylactic type reaction which is likely to come on within seconds or minutes after the injection of penicillin. Fortunately, no such reactions were encountered in this series of cases. The delayed reactions also tended to be more prolonged, although the observed differences here were not significant and were influenced by other therapy.

CONCLUSIONS

1. Experimental conditions have been defined for evaluating procedures influencing the incidence of penicillin reactions.

2. A controlled study failed to show that an oral and/or parenterally administered antihistamine produced any significant effect on the incidence of

delayed or severe penicillin reactions, although there was a reduction of early reactions, particularly those of an urticarial type. These findings are in accordance with the known pharmacologic properties of the antihistamines.

3. Penicillin reactors had a significantly higher incidence of moderate or severe local reactions than nonreactors. Delayed reactions were severe more often than early ones and occurred relatively less often in patients who had received penicillin within one year. Numerous other attempted correlations failed to be statistically significant.

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IMMUNOLOGIC AND CLINICAL STUDIES ON ALLERGENIC FUNGI

I. Purification of an Antigen From Alternaria

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INTEREST in fungi as a cause of allergy has increased since Feinberg and his associates[1] and others[2, 3] called attention to their importance about twenty years ago. Next to pollen, fungi constitute the most common group of causes of respiratory allergy. For studying the antigenic relationship of various molds and for other immunologic investigations, it is important to use a well-defined, preferably pure antigen. This is a report on the purification of an antigen of alternaria, the most prevalent cause of mold allergy.

A procedure for fractionation of alternaria extract was developed. The products resulting from each step were tested by precipitation with rabbit antiserum and by skin reaction on allergic persons. The purified fractions were evaluated serologically and clinically. Structural studies will be reported in a subsequent paper.

FRACTIONATION OF ALTERNARIA

Alternaria was grown by the method of LeBeau[4] on a modified Steinberg-Thorne medium in which sucrose was the only source of carbon and sodium nitrate the only source of nitrogen. The pellicles were drained of excess fluid over a Buchner funnel, dried at room temperature, and homogenized in a Waring blender. The mold powder was swirled at room temperature for twenty-four hours with twenty volumes of 0.85 per cent sodium chloride solution containing Merthiolate 1:10,000. The extract was obtained by filtration. The fractionation procedure developed is illustrated in Fig. 1. All steps were performed at 1° C. A dialyzable fraction was obtained by dialysis of the extract against an equal volume of water in rolling tubes. The nondialyzable residue was further dialyzed against two changes of 15 liters each of water. It was then deproteinized by the procedure of Sevag.[5] A precipitate was obtained from the deproteinized solution by addition of an equal volume of

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0.2N uranyl acetate. The precipitate was washed twice with water and then redissolved in 1N hydrochloric acid, using one-tenth of the volume from which the precipitate had been originally obtained. Uranyl chloride was removed by dialysis against two changes of 15 liters each of water. The dialyzed, redissolved precipitate was concentrated further by centrifugal ultrafiltration. This was accomplished by the use of a Boerner filter,[6] and 44 mm. wide Visking dialysis tubing (Fig. 2). An asbestos filter pad (*C*) was placed in the filter. The dialysis tubing was closed at one end by three knots and the sac thus formed was introduced into the receptacle (*A*) of the filter. The dialysis tubing was compressed in an accordion-like fashion (*E*) to increase the available surface area, and the open end was inverted over the outside of the receptacle. The cord formed at the closed end of the sac (*F*) was also extended to the outside of the receptacle of the filter underneath the open end of the sac.

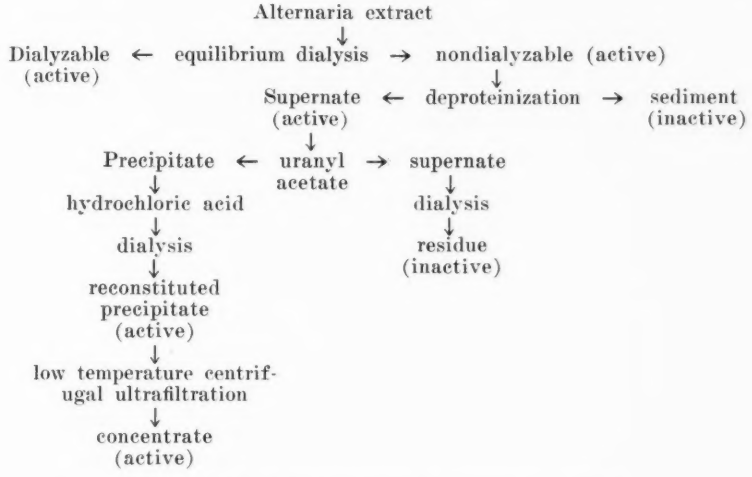

Fig. 1.—Flow sheet of fractionation of alternaria extract.

Both the open end and the underlying closed end of the dialysis sac were fastened to the outside of the receptacle by means of a rubber band (*H*). The dialysis bag was then filled with the redissolved precipitate and the assembly was covered with Parafilm (*G*) attached with another rubber band (*H'*) and centrifuged at 1° C. for two hours at 1,300*g* exerted at the level of the filter pad.

CONTROL OF FRACTIONATION PROCEDURE

The fractions obtained in each step were tested for specific reactivity on sensitive human skin and for precipitability with rabbit antiserum.

Skin testing was done by scratches with serial threefold dilutions of the fractions. The end point adopted was the highest dilution giving a wheal and erythema reaction.

The amount of material precipitating with rabbit antiserum was estimated by the method of Heidelberger and Kendall,[7] which involves the addi-

tion of an excess of calibrated antiserum to a small amount of antigen. Anti-alternaria serum was prepared as follows: Six-month-old Flemish Cross rabbits, weighing 4 kg., received four simultaneous intramuscular injections of 1 ml. each, containing 40 mg. of alternaria in extract in Freund's complete adjuvants[8, 9] in the proportions employed in reference 10. Sixty milliliters blood was obtained from the ear artery each week, beginning five weeks after

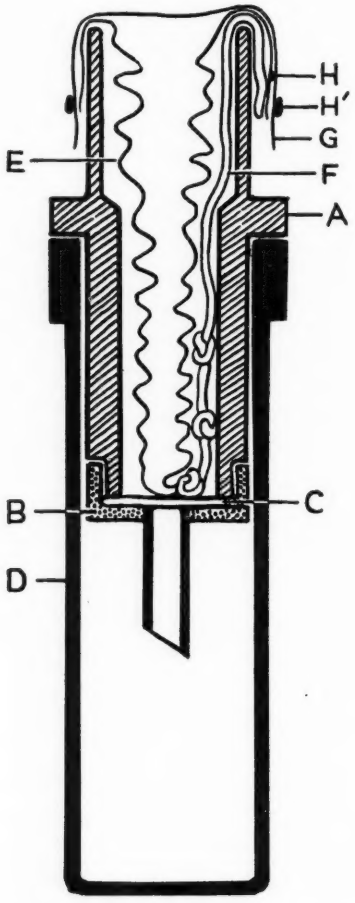

Fig. 2.—Assembly for centrifugal ultrafiltration. *A*, Receptacle tube; *B*, lower part of filter; *C*, filter pad and supporting screen; *D*, silver-plated metal receiving tube; *E*, dialysis tubing arranged in accordion-like fashion; *F*, closed end of dialysis tubing knotted to a cord; *G*, Parafilm covering; *H*, *H'*, rubber bands.

immunization. Triplicate mixtures of the various antigen preparations and excess antiserum were incubated at 37° C. for two hours, left at 1° C. for eleven days, and centrifuged at 1,000*N* at 1° C. for twenty minutes. The precipitates were washed three times with 7.0 ml. portions of 0.85 per cent sodium chloride solution at 1° C. Their protein contents were estimated by the method of Heidelberger and MacPherson,[11] standardized by nesslerization.[12] Since it was not known whether all the purified material was precipitated by

antiserum, absolute values could not be obtained with this procedure. The results indicate only the degree of change in the relative amount of total solids precipitable by antiserum during the purification procedure. Normal rabbit serum and serum from immunized rabbits obtained earlier than fourteen days after immunization failed to precipitate with crude alternaria extract, the nondialyzable principle, or the reconstituted uranium precipitate.

CHARACTERIZATION OF THE ANTIGENS FROM ALTERNARIA

Table I shows that the uranium-precipitated material, after reconstitution and ultrafiltration, contained only 1/93 of the solids of the original extract. At the same time it was 82 times more concentrated. The precipitability with rabbit serum of the reconstituted uranium precipitate was 84 times as much as that of the original extract when the amount of immune precipitate was compared to the total solids of the solutions. Since total solids were reduced by a factor of 93 it seems that most of the specifically precipitated antigen went into the uranium-precipitated fraction. The residual fraction from

TABLE I. CONTROL OF FRACTIONATION OF ALTERNARIA EXTRACT

	ORIGINAL EXTRACT	DIALYZABLE	NONDIALYZABLE	DEPROTEINIZED	URANIUM PRECIPITATE RECONSTITUTED	CONCENTRATED	RESIDUAL
Degree of purification*	1		18	21	93	93	
Degree of concentration†	1	0.5	0.91		8.2	82	0.6
Relative weight fraction precipitable with rabbit antiserum‡	1	Inhibits	17		84		No precipitate
Scratch test titer:							
Subject 1	1:4,800	1:180	1:540	1:1,620	1:540	1:1,620	1:60
Subject 2	1:14,000		1:180		1:4,860		1:40
Subject 3	1:4,800		1:4,860		1:1,620		
Minimum total solids reacting in scratch test (μg)§	8	200	4	1	0.7	0.2	
	2		11		0.08		
	8		0.4		0.2		

*Ratio of weight of total solids of the original extract to weight of total solids of fractionation product adjusted to the volume of original extract from which fraction was derived.

†Ratio of volume of original extract to the volume of fractionation product.

‡Calculated by dividing the ratio of the amount of precipitate nitrogen obtained in antibody excess from fractionation product to its total solids by the ratio of the amount of precipitate nitrogen obtained in antibody excess from the original extract to its total solids.

§Using 0.05 ml. for each test.

which the uranium precipitate was obtained was free of material precipitating with specific rabbit antiserum. When the uranium-precipitated reconstituted material was made up to the volume of the original extract from which the product was obtained and tested for activity on allergic human skin, titers of 1:540 to 1:4,800 were obtained. This is less than the titers of the original extract (1:4,800 to 1:14,000), indicating that only about one-third of the reactivity of the original extract was obtained in the purified fraction. The residual fraction was practically free of antigen as determined by scratch tests.

The dialyzable fraction failed to precipitate with rabbit antiserum. Nevertheless, it was capable of reacting on scratch tests. The fraction of

scratch test activity of the original extract which went into the dialysate perhaps may account, in part, for the loss of skin reactivity upon purification of the nondialyzable fraction. The following considerations may also support the possibility that much of the skin reacting material was lost in the dialysate and that only little was lost in the residual fractions:

(1) The residue, after uranium precipitation, was free of scratch activity.

(2) The scratch reactivity did not decrease appreciably when the material was deproteinized. Hence, the protein fraction did not contain significant activity. Only a small amount of denatured protein was obtained by deproteinization.

(3) Practically all antigenic reactivity with rabbit serum was obtained in the uranium-precipitated reconstituted fraction.

(4) The uranium-precipitated reconstituted fraction had no less skin reactivity than the crude nondialyzable fraction. Most of the skin reactivity was lost in the first step of dialyzing the original extract.

Gel diffusion tests[13] with alternaria proved unsatisfactory, as the antigen caused a high degree of nonspecific haziness upon diffusion into the agar. Experiments are in progress to resolve this difficulty. Precipitin analysis, on the other hand, permitted a presumptive evaluation of the purity of the antigen in its reaction with rabbit antiserum. Varying quantities of dialyzed crude alternaria extract (nondialyzable fraction) concentrated by centrifugal ultrafiltration were mixed with constant amounts of rabbit antiserum in triplicates. The tubes were incubated at 37° C. for two hours, left at 1° C. for eleven days, and centrifuged at 1,000*g* at 1° C. for twenty minutes. The supernates were collected and the triplicate precipitates from some of the antigen concentrations employed were washed three times with 7.0 ml. portions each of 0.16 normal sodium chloride solution and analyzed for their protein contents.[11] These represented antibody, since the antigen did not give a significant reaction with the Folin-Ciocalteau reagent. The supernates were tested for both excess antigen and excess antibody. The test for excess antigen involved addition of equal volumes of antialternaria rabbit serum to the supernates, and the test for excess antibody involved addition of equal volumes of antigen in concentrations of 3.7 mg. and 29.4 mg. total solids per milliliter. The higher concentration of antigen was employed in order to facilitate addition of a sufficient quantity of any antigenic component that may be present in the crude extract in trace amounts only. In the original precipitation the antibody to such an antigenic component may not have been completely removed by the quantities of dialyzed crude extract employed and, consequently, subsequent addition of a larger quantity of antigen may result in precipitation.[14]

Table II shows that 14.7 mg. of dialyzed crude antigen was equivalent to the antibody of 1 ml. of antiserum; neither antigen nor antibody was left in solution. Larger amounts of antigen precipitated in antigen excess and smaller amounts in antibody excess. If the dialyzed crude antigen were composed of several antigenic components, it would have been probable that the amount of dialyzed crude extract sufficient to remove in equivalence all the antibody to one of its antigenic components would not represent an equivalence proportion to another antigenic component.[15] Occurrence of a true equivalence in the data of Table II is compatible, therefore, with the existence of only one antigenic component in the dialyzed crude alternaria extract. Consequently, the purification of this crude extract apparently removed only inert material, and did not separate different antigenic components.

TABLE II. REACTION OF ANTIALTERNARIA RABBIT SERUM WITH DIALYZED CRUDE EXTRACT OF ALTERNARIA

TOTAL SOLIDS OF CRUDE DIALYZED EXTRACT ADDED PER MILLILITER OF ANTISERUM (MG.)	ANTIBODY N PRECIPITATED PER MILLILITER OF ANTISERUM (μG)	EXCESS ANTIGEN	EXCESS ANTIBODY	
			TESTED WITH 3.7 MG. DIALYZED CRUDE EXTRACT PER MILLILITER SUPERNATE	TESTED WITH 29.4 MG. DIALYZED CRUDE EXTRACT PER MILLILITER SUPERNATE
1.8	12	-	+	+
2.3	17	-	+	+
3.7		-	+	+
4.7	28	-	+	+
7.3		-	+	+
9.3	62	-	+	+
14.7		-	-	-
29.4		+	-	-
58.8		+	-	-
118.0		+	-	-
233.0		+	-	-
466.0		+	-	-

In another experiment, antialternaria rabbit serum was absorbed with an equivalence amount of uranium-precipitated reconstituted precipitate. The absorbed serum was reacted with each of the fractions shown in Fig. 1; in no case did a precipitate develop. These results further indicate that the uranium-precipitated reconstituted fraction contained all the antigen of the original extract capable of precipitation with rabbit antiserum.

In order to determine whether the antigen reacting on the human skin and that reacting with rabbit antiserum are identical, the purified uranium-precipitated and reconstituted principle was mixed with an excess of rabbit antiserum, incubated at 37° C. for two hours, left at 1° C. for eleven days, and centrifuged at 1,000*g* at 1° C. for twenty minutes. Dilutions of the supernate were tested on the allergic human skin by scratches along with dilutions of mixtures of the purified principle and 0.85 per cent sodium chloride solution. The data of Table III show that the precipitation with rabbit serum absorbed practically all the scratch test reactivity of the purified principle.

TABLE III. ABSORPTION OF SKIN REACTIVITY OF NONDIALYZABLE RABBIT SERUM

DILUTION OF URANIUM-PRECIPITATED ANTIGEN	SCRATCH TEST REACTION	
	UNABSORBED	ABSORBED WITH RABBIT ANTISERUM
1:60	++	+
1:180	++	+
1:540	+	-
1:1,620	+	-
1:4,860	+	-
1:19,560	+	-

These data indicate that most of the scratch-reactive material in the purified nondialyzable fraction is identical with the antigen precipitated with rabbit antibody.

The small amount of scratch test reactivity remaining after absorption with rabbit antiserum may be due to any of the following causes:

(1) It may reflect nonprecipitating antibody-antigen complex.[16]

(2) Precipitating antibody-antigen complex has a small but definite solubility.[17] A small amount of antigen thus may have remained in solution along with some precipitating antibody. This interpretation requires the assumption that the precipitating antibody retained in solution does not block the skin reactivity of the antigen.

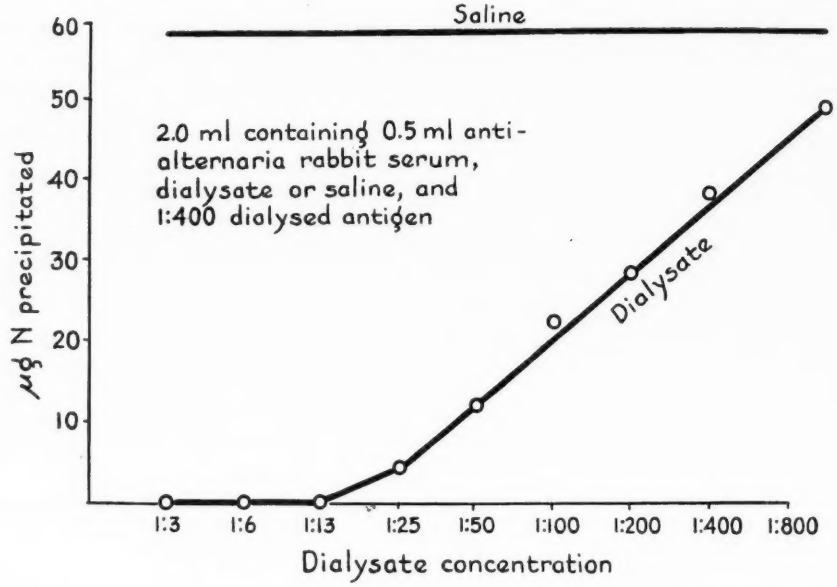

Fig. 3.—Effect of dialyzable principle on precipitation of nondialyzable principle and rabbit antiserum.

(3) The uranium-precipitated reconstituted material may contain a trace amount of a skin-reacting antigen which differs from the antigen precipitated by rabbit antiserum. This antigen may elicit a skin reaction without being able to precipitate with rabbit antiserum. Alter-

natively, it may be capable of precipitation with rabbit antiserum but may be available in traces so small that the amount of complex formed with precipitating antibody is less than the solubility of the precipitate. Again this interpretation can hold only if the precipitating antibody retained in solution does not block the skin reactivity of the antigen.

Experiments to resolve these interpretations are in progress.

The dialyzable material which formed no precipitate with rabbit antiserum was capable of inhibiting specifically the precipitation of the nondialyzable antigen and rabbit antiserum. In the experiment shown in Fig. 3, triplicates of 0.5 ml. of antialternaria rabbit serum were mixed with 0.85 per cent sodium chloride solution and sufficient dialyzed antigen (nondialyzable fraction) to give a 1:400 concentration in a total volume of 4.0 ml. This mix-

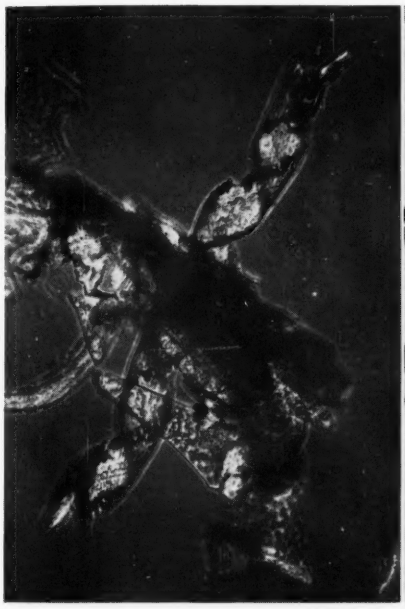

Fig. 4.

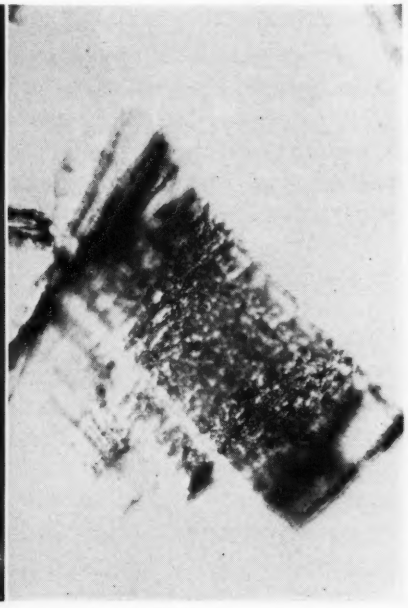

Fig. 5.

Fig. 4.—Phase micrograph of purified principle. (Magnification, ×60; reduced ⅓.)
Fig. 5.—Phase micrograph of purified principle. (Magnification, ×60; reduced ⅓.)

ture precipitated in slight antibody excess. The tubes were incubated at 37° C. for two hours, left at 1° C. for eleven days, and centrifuged at 1,000*g* at 1° C, for twenty minutes. The precipitates were washed three times at 1° C. with 7.0 ml. portions of 0.85 per cent sodium chloride solution each. Their protein N contents were found to be 58 μg. When increasing concentrations of the dialyzable material were substituted for the sodium chloride solution, decreasing amounts of nitrogen were precipitated. Fifty per cent inhibition occurred with a 1:100 concentration of dialyzable material. A 1:13 concentration inhibited precipitation completely. The dialyzable material failed to in-

hibit the precipitation of crystalline bovine plasma albumin with anticrystalline-bovine-plasma-albumin rabbit serum. These data indicate that the dialyzable material is a hapten of specificity similar to the nondialyzable complete antigen.[18] Probably it differs from it only in molecular size.

The dried, purified, nondialyzable material formed characteristic fibrils (Figs. 4 and 5). The individual fibrils seemed to aggregate in the form of plates. On the sides of these plates the ends of the fibrils can be seen. These fibrils are highly soluble in water. The substance contains acid radicals precipitable by uranyl ion, but it cannot be precipitated with barium, cobalt, copper, lead, mercury, silver, or zinc ions. It cannot be precipitated isoelectrically at low ionic strength and does not precipitate in 25 per cent v/v ethanol at –5° C. at any pH. It is soluble in 7.5 per cent trichloroacetic acid, in 50 per cent v/v dioxane, and in 0.05M tannic acid. It is slightly soluble in 50 per cent v/v ethanol and fairly insoluble in acetone and acetonitrile. It is salted out by ammonium sulfate with difficulty.

The nitrogen content of the purified nondialyzable material is 0.57 per cent as determined by nesslerization.[12] Biuret and ninhydrin tests are negative. The material contains primary or secondary amino groups, as indicated by conversion to dithiocarbamate.[14] It is free of phosphorus, as determined by the method of Fiske and Subbarow,[20] and seems to contain no reducing sugar even after acid or alkali hydrolysis at 120° C. for two hours. The material, therefore, is not a typical polysaccharide, but a positive Molisch test may suggest the presence of five or six carbon chains. Tests for glucuronic, gluconic, and several other common aliphatic carboxylic acids[21] are negative. The acidic groups are not due to a sulfated polysaccharide, since the medium on which the alternaria was grown contained only traces of sulfur.[4] The material reduces iodine at a neutral pH without destruction of serologic specificity, perhaps suggesting a double or triple bond in a carbon chain. The purified material contains traces of uranium (estimated in a well scintillation gamma counter) which could not be removed without destruction of antigenic activity.

These preliminary chemical data do not permit a conclusion about the structure of the purified material. More extensive chemical procedures are necessary for the characterization of this antigen. However, the data up to date have suggested the nature of several simple chemical compounds that may enter into the formation of the molecule of the active principle of alternaria. Some of these compounds have been used successfully as specific haptens to inhibit the reaction of alternaria and rabbit antiserum. The information gained by these hapten inhibition studies will be reported in a subsequent paper of this series.

DISCUSSION

The fractionation procedure developed resulted in a ninety-three-fold reduction of solids and an eighty-two-fold concentration of the "complete antigen"[18] from alternaria. Although the material appears in the form of crystal-like fibrils, so far proof of purity is lacking. It was possible to concentrate all

the "complete antigen" of alternaria in a single fraction. The dialyzable fraction, however, contained a hapten with a specific activity similar to the complete antigen. No antigenic activity was contained in the other fractions. Practically all the specific groups reacting on the allergic skin were found identical to those reacting with rabbit antiserum.

It is interesting that the antigen is not a protein or a polypeptide, differing in this respect from many other immediately reacting allergens. The hapten gave immediate reactions on the allergic skin, although it was unable to precipitate with rabbit serum.

It is often observed that desensitization of patients with mold allergy is difficult to attain. Desensitization procedures to other antigens, such as ragweed, probably include some form of immunization, perhaps resulting in a production of blocking antibody.[21] The practice of repeat injections of antigen in desensitization is likely to elicit anamnestic responses.[22, 23] Recall reactions, however, have so far been demonstrated only with proteins. They seem to be absent in immunization with polysaccharides.[24] Perhaps the nature of the antigen from alternaria may be such that anamnestic reactions cannot be elicited. This may offer one of the possible explanations for the difficulties encountered in the treatment with mold extracts.

Mold allergy may persist in patients for very long periods, even though they had no extensive contact with the antigen for years. Immunity to a single injection of polysaccharides, like pneumococcal polysaccharide, may persist much longer than that to proteins,[24] although recent findings appear to explain why antibody production also may continue for a reasonably long time after the administration of a single dose of protein antigen.[25] The fact that the complete antigen of alternaria is not a protein may help to explain the observed persistence of mold allergy.

SUMMARY

A dialyzable and a nondialyzable principle were obtained from alternaria grown on a synthetic medium. The antigen in the nondialyzable fraction was purified by deproteinization, precipitation as the uranyl salt, and low-temperature centrifugal ultrafiltration. The nondialyzable fraction reacted by skin test in allergic individuals and was precipitated with rabbit antiserum. The dialyzable fraction also reacted on skin tests. It failed to precipitate with antiserum but acted as a hapten in specifically inhibiting precipitation of the nondialyzable fraction.

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STUDIES IN PENICILLIN HYPERSENSITIVITY

III. The Influence of Parenterally Administered Antihistamines on Contralateral Passive Transfer Reactions to Penicillin

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THE admixture of antihistamine drugs with penicillin has been recommended for the prevention of allergic reactions to the antibiotic. Although some allergic reactions to penicillin have been prevented by this procedure,[1-3] it has not been established that the most serious form of penicillin allergy, the immediate allergic shock or anaphylactic reaction, can be suppressed by antihistamines. These shock reactions develop with such rapidity and explosiveness that a fatality may occur within a few minutes after an injection of the penicillin. The problem of whether or not antihistaminic drugs can prevent allergic reactions of this type was studied experimentally by us, and our results are presented in this communication.

It was considered hazardous to attempt to investigate this problem directly on extremely sensitive patients, many of whom have circulating reagins to penicillin in their blood.[4] For this reason, we resorted to passive transfer studies, which were performed on normal subjects with the use of a reagin-bearing penicillin-sensitive serum obtained from a patient who had experienced two severe immediate allergic shock reactions following injections of the antibiotic.[5]

METHOD

Two sites were sensitized on the left arms of five normal subjects by the intracutaneous injection of approximately .05 ml. of undiluted serum and of a 1:4 saline dilution of the serum. After seventy-two hours, 1 ml. of a procaine penicillin G suspension, containing 300,000 units, was injected intramuscularly into the right arm of each subject. This type of penicillin and dosage were selected because they are commonly used in practice. A reaction, starting with itching and erythema and followed by wheal formation, flared up at the sensitized site on the opposite arm approximately five to ten minutes after the injection. Readings were made when the reactions reached their height from fifteen to thirty minutes after onset. The amounts of wheal and erythema were graded separately.[6]

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This phenomenon of excitation of a distant, passively sensitized site by antigen conveyed to it by way of the blood stream is particularly suitable for the present study, as it has been described by Walzer[7, 8] as the equivalent of a constitutional reaction in a nonsensitive subject. Thus, experimentally and in a miniature way, one may reproduce and measure one of the common outstanding features of the allergic constitutional reaction, the urticarial reaction, without danger to the patient or recipient. The phenomenon has been called "Fernauslösung" by Jadassohn[9] and "contralateral passive transfer" by Chant and Gay.[10] Most of the penicillin-sensitive sera studied by us characteristically demonstrated this capacity to flare up.

After an interval of several weeks the experiments were repeated on the same recipients, but at this time 10 mg. of an antihistamine (Chlor-Trimeton) was mixed with the procaine penicillin just prior to injection. The resulting reactions were compared with those obtained in the initial experiments.

In order to determine the smallest dose of the antibiotic needed to elicit a contralateral passive transfer reaction, a similar series of experiments was performed on nine sensitized subjects who were injected with 1 ml. of a solution of crystalline potassium penicillin G, containing 5, 50, or 500 units. The effectiveness of the admixture of 10 mg. of antihistamine with the minimal exciting doses of penicillin in the prevention of the local urticarial reactions was then studied.

RESULTS

The addition of 10 mg. of the antihistamine, Chlor-Trimeton, to 300,000 units of procaine penicillin G failed to prevent the flaring of sites sensitized with undiluted serum or with a 1:4 dilution (Table I). Moreover, with this therapeutic dose of antigen there was no evidence of a consistent depressing

TABLE I. EFFECT OF THE ADMIXTURE OF ANTIHISTAMINE WITH THERAPEUTIC DOSE (300,000 UNITS) OF PROCAINE PENICILLIN G ON CONTRALATERAL PASSIVE TRANSFER REACTIONS

	WITHOUT ANTIHISTAMINE				WITH ANTIHISTAMINE (10 MG. CHLOR-TRIMETON)			
	REACTIONS ON SITES SENSITIZED WITH				REACTIONS ON SITES SENSITIZED WITH			
	UNDILUTED SERUM		SERUM 1:4		UNDILUTED SERUM		SERUM 1:4	
SUBJECT	WHEAL	ERYTHEMA	WHEAL	ERYTHEMA	WHEAL	ERYTHEMA	WHEAL	ERYTHEMA
1. P. G.	+++	+++	±	+	++	++±	+	+
2. M. B.	+++±	++++	+++	++++	++	+++	+	+
3. M. F.*	+++	-	+	-	++	-	+	-
4. E. L.	++	+++	+	++	++	+±	+	+
5. D. W.*	++	-	±	-	+++	-	+	-

*Subjects 3 and 5 were Negroes and erythemas were not recordable.

effect of the antihistamine on the intensity of the contralateral passive transfer reaction. Of the ten whealing reactions that developed after the administration of the antihistamine-penicillin mixture, four were smaller, two were larger, and four were equal when compared to the corresponding reactions

obtained with procaine penicillin alone. The erythematous components of these reactions were usually diminished but not suppressed by the antihistamine.

The minimal exciting dose of potassium penicillin G needed to induce a contralateral passive transfer reaction was found, in different subjects, to range approximately between 50 and 500 units (Table II). When minimal exciting doses of antigen were used for testing on nine subjects, the contralateral passive transfer reaction on an undiluted serum site was completely suppressed in only one instance (Case 4) by 10 mg. of the antihistamine. The wheal reaction on this subject had been only minimal (±) on the undiluted serum site when no antihistamine was used. In the remaining eight subjects, positive reactions occurred despite the admixture of antihistamine with the minimal exciting doses of penicillin prior to injection. However, an inhibiting effect, resulting in a slight reduction in size of the wheal and erythema, was apparent in the majority of instances. In Case 1, a minimal wheal (±) was suppressed and a two-plus (++) erythema was reduced but not prevented.

TABLE II. EFFECT OF THE ADMIXTURE OF ANTIHISTAMINE WITH MINIMAL EXCITING DOSES OF POTASSIUM PENICILLIN G ON CONTRALATERAL PASSIVE TRANSFER REACTIONS

		WITHOUT ANTIHISTAMINE REACTIONS ON SITES SENSITIZED WITH				WITH ANTIHISTAMINE (10 MG. CHLOR-TRIMETON) REACTIONS ON SITES SENSITIZED WITH			
		UNDILUTED SERUM		SERUM 1:4		UNDILUTED SERUM		SERUM 1:4	
SUBJECT	UNITS OF PENICILLIN	WHEAL	ERY-THEMA	WHEAL	ERY-THEMA	WHEAL	ERY-THEMA	WHEAL	ERY-THEMA
1. P. G.	50	±	++	0	0	0	+	0	±
2. L. R.	500	+	+++	0	+	+	++	0	±
3. M. T.	50	+++	+++	+	++	+	+++	0	++
4. R. P.	50	±	++	0	0	0	0	0	0
5. M. E.	50	+	++	0	0	+	+	0	0
6. M. S.	50	0	+++	0	+	0	+	0	0
7. F. B.	500	++	++	0	++	+	++	0	0
8. R. G.	50	0	+++	0	+	0	++	0	0
9. F. F.	50	+±	++	+	++	±	++	0	++

In Cases 2 and 5, one-plus (+) wheal reactions were not suppressed by antihistamine, nor did this occur in the remaining instances where the original reaction was greater than one-plus (+). With the exception of Case 4, previously mentioned, no erythema of ++ intensity or greater, was completely suppressed by the antihistamine.

Only two of the 1:4 diluted serum sites (Cases 3 and 9) gave positive wheal reactions with the minimal exciting doses, and these were only one-plus. While antihistamine suppressed both of these wheal reactions, it failed to prevent the erythema formation on these sites. Of the four cases in which the original reactions at the 1:4 dilution sites consisted only of erythema, three were suppressed by the antihistamine.

DISCUSSION

In the present study, the contralateral passive transfer reaction was interpreted as a local expression of a "constitutional reaction in the normal recipient," which, in the sensitive patient such as the one who provided

the serum, undoubtedly would have manifested itself as a severe generalized constitutional reaction, possibly ending in a fatality. In the normal passively sensitized recipient, the injected antigen reaches the sensitized site via the circulation and reacts locally with the reagin to produce an isolated urticarial reaction, whereas in the sensitive patient, with reagins to penicillin in his circulation and tissues, a severe, immediate, generalized allergic reaction develops under the same circumstances.

The mixing of antihistamine with the average therapeutic dose of penicillin had no pronounced or consistently deterrent effect on the development of a contralateral passive transfer reaction. Even with minimal exciting doses of penicillin, the antihistamine failed to prevent reactions at undiluted serum sites in eight out of nine substitutes.

These experiments have demonstrated that the admixture of 10 mg. of Chlor-Trimeton with penicillin could not completely prevent contralateral reactions induced by doses of penicillin at or slightly in excess of threshold levels. In establishing the minimal exciting dose of penicillin in these experiments, a dilution factor of ten had been employed for the antigen. It is apparent, therefore, that antihistamine, in the dosage generally recommended, can have no pronounced prophylactic effect against immediate constitutional reactions when therapeutic doses of penicillin, which range from 600 to 6,000 times more than the minimal exciting doses, are used.

There is, therefore, little reason to expect that the admixture of an antihistamine drug with penicillin would prevent a severe, immediate, allergic reaction from developing in a markedly sensitive patient with reagins to penicillin. The clinician should not be misled into a false sense of security by the prophylactic effects of antihistamines which have been reported in relation to other nonreaginic forms of penicillin allergy. Such benefits are not to be anticipated in the more serious and dangerous immediate reaginic type of sensitivity under discussion here.

CONCLUSIONS

1. The addition of an antihistamine drug to a *therapeutic* dose of penicillin had no significant deterrent effect on the development of contralateral passive transfer reactions on sites passively sensitized with a serum containing reagins to penicillin.

2. Little preventive action of antihistamine could be demonstrated in similar experiments with this serum when *threshold* doses of penicillin were used.

3. On the basis of the evidence obtained with this serum, it seems unlikely that the admixture of antihistamine drugs with penicillin will prevent the severe, immediate, allergic shock or anaphylactic reaction from developing in the extremely sensitive person with reagins to this antigen.

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THE EFFICIENCY OF MECHANICAL FILTERS AND AIR CONDITIONERS IN THE REMOVAL OF RAGWEED POLLEN AND FUNGUS SPORES

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SCHEPPEGRELL,[1] in 1924, described an air-washing apparatus for the removal of air-borne pollen. In 1925 Leopold and Leopold[2] studied the effect of air filtration under controlled conditions of environment, temperature, and humidity on bronchial asthma, allergic coryza, and hay fever. Cohen[3] noted in 1926 that, when patients with hay fever were placed in a pollen-free room, some relief was given in a few minutes and that after six to eight hours there was complete relief.

Peshkin and Beck[4] pointed out in 1930 and Rappaport, Nelson, and Welker,[5] in 1932, that air filtration that removes most of the pollen will relieve symptoms of hay fever and that this method is particularly valuable in those cases not benefited by the usual methods of treatment. Beck,[6] also in 1932, reported that in almost 80 per cent of properly selected cases patients can find adequate relief through the use of pollen-free air produced by a filter of tested high efficiency. The air filter used was reported as producing 100 per cent pollen-free air. However, details as to tests conducted are lacking. Gay,[7] in 1933, studied the effect of air filtration and cooling in the treatment of hay fever and pollen asthma. The patients who had ragweed hay fever were free from symptoms in one hour. The patients with pollen asthma had moderate wheezing for the first fifteen hours after admission to the air-conditioned atmosphere. The symptoms were quite mild, however, as compared with the discomfort previous to admission to the air-conditioned atmosphere. The relief given was attributed primarily to air filtration.

Nelson, Rappaport, and Welker,[8] in 1933, reported experiments conducted on two special air conditioning units. Slides were exposed in the protected ward and outdoors. A comparison of these slide counts indicated an efficiency of 98.2 per cent in terms of air-borne pollen. The conclusions were drawn that no pollen filter on the market at that time could claim 100 per cent efficiency for pollen, but that filters that remove all but traces of pollen will relieve symptoms of hay fever. Cooled filtered air without dehumidification did not give as prompt or as great relief from symptoms of hay fever as uncooled filtered air.

Kahn and Grothaus,[9] in 1933, reported the successful use of a homemade unit that reduced the air-borne pollen by about 95 to 98 per cent. Vaughan

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and Cooley[10] used a standard air conditioning unit as a means of removing pollen and spores. Petrolatum-coated slides were exposed in the air-conditioned room, in a control room without air conditioning, and on an outdoor window sill. They found that appreciable numbers of both air-borne pollen and spores were removed by the water of condensation from the air conditioner.

Criep and Green,[11] in 1935, apparently were the first to study the use of an electrostatic air cleaner as an aid in the treatment of hay fever and bronchial asthma. The efficiency of this method in a series of experiments was compared with that of a glass wool mechanical filter. Pollen was introduced into the unit under test, and exposed slides were used to determine the efficiency. The average efficiency of the mechanical filter was found to be 96.5 per cent, while that of the electrostatic air cleaner was reported as 100 per cent efficient in removing pollen from the air. Barnes and Rooks,[12] in 1946, by the use of two window type pollen filtering units, were able to set up and maintain through the summer an entire home comparatively free of pollen.

Friedlaender and Friedlaender,[13] in 1954, studied the effectiveness of a portable electrostatic precipitator in the elimination of environmental allergens and in the control of allergic symptoms. A series of pollen experiments utilizing exposed petrolatum-coated slides to determine efficiency indicated the value of these units in clearing the air of experimentally introduced and naturally occurring pollen. These units, when placed in bedrooms and hospital rooms of selected patients suffering from ragweed hay fever and asthma, produced prompt and sustained relief in most patients. The electrostatic air cleaner also was found very effective in the treatment of certain asthmatic patients allergic to household dusts.

STATEMENT OF PROBLEM

This report is concerned with a study of the efficiency of mechanical filters and air conditioners in the removal of air-borne ragweed pollen and certain fungus spores as determined by a volumetric sampler.

It is apparent that in the use of a good mechanical filter a sufficient amount of air-borne ragweed pollen can be removed so that air filtration will give relief in a short time to patients suffering from ragweed hay fever, while patients having asthma as a result of a ragweed sensitivity respond much more slowly to filtered air treatment. However, the figures (in per cent) reported representing the efficiency of the unit under test should be questioned.

In all the studies reviewed, petrolatum-coated slides were exposed for from a few minutes up to several days to measure efficiency. The effectiveness of the unit under study was determined for naturally occurring pollen or, in some instances, by experimentally introduced pollen. Slide counts indoors were compared in some studies with slide counts outdoors, but these slide counts are not comparable due to marked differences in sampling conditions. The pollen catch in terms of an exposed slide would depend primarily upon such factors as the settling rate of pollen, the effect produced by air turbulence, and air velocity.

In other instances the slide was exposed directly in the air stream from the test unit, in which case only a fraction of the pollen liberated from or passing through the instrument would be recovered.

The efficiency of air filtration methods, as measured by a suitable volumetric sampler during the actual ragweed pollen season in a high incidence area, should more nearly approximate the actual dosage of pollen to which the individual is exposed when indoors. Because of the importance of hormodendrum and alternaria spores in the production of allergic symptoms, it was decided to include these in the study.

PROCEDURE

The sampler described by Stenburg[14] was selected for this problem because of familiarity with this sampler, its simplicity in operation, and the permanency of records. In a previous study[15] this sampler was reported as having a 76 per cent sampling efficiency in terms of commercially collected ragweed pollen *(Ambrosia trifida)*. The sampling efficiency of this instrument for hormodendrum and alternaria spores is not known, but it must be less than for pollen because of their smaller size and because sampling is by impingement to a moving strip of adhesive tape.* All samples, then, in terms of efficiency, represent the minimum exposure of the individual both to ragweed pollen and to hormodendrum and alternaria spores. However, all readings were taken during the height of the ragweed season during the months of August and September and, inasmuch as it has been reported[15] that 56 per cent of the pollen collected over a twenty-four-hour period was collected from 10:00 A.M. to 1:00 P.M., all air samples were taken during this same time interval.

The rate of air flow used in all air samples was 0.5 cubic feet per minute, and 0.5 cubic yard of air was sampled in each instance. This sampling rate approximates the volume of air inspired by an adult during normal activity.

Air samples were taken at the same height indoors and outdoors, in each instance at a height of four feet above the floor or ground. The sampler was centrally located in the test area indoors, but was kept outside any direct air stream from the test unit. Outdoor air samples were taken at the same side of the home or building representing the air intake into the unit and immediately followed the air sample taken indoors.

Four different locations were used; two were protected by commercial window type filtering units supplying only filtered air, manufactured by the same company, and two by standard air conditioning units manufactured by different companies. A total of approximately 100 air samples were taken at these four widely separated locations, and all counting and identification was carried out under high-power magnification.

DISCUSSION AND RESULTS

An inspection of each unit was carried out prior to its operation to be certain that it was installed in such a manner as to minimize air leakage, that the

*Scotch cellophane tape, ¾ inch by 300 inches (Minnesota Mining & Mfg. Co., Saint Paul 6, Minnesota).

unit contained a new, clean filter which was properly fitted in the unit, and that there was no unnecessary vibration of the unit which might affect the pollen retention of the filter.

Location No. 1.—Filtered air was supplied to a single room, the bedroom, by installation of a commercial unit* in one of the windows of this room. No special precautions were taken from the standpoint of pollen or spores except that the other windows in this room were kept tightly closed. The door from this room on the second floor was open many times to the rest of the home during operation of the unit.

The patient, a young boy, had a ragweed sensitivity for a number of years, with symptoms of both hay fever and asthma. He was having a great deal of difficulty, especially at night, even though undergoing treatment. His sleep remained undisturbed generally after installation of the unit, although asthmatic symptoms at night were not entirely eliminated.

The mean ragweed dosage, as determined by the volumetric sampler outdoors at this location, was 218 per cubic yard of air. This was reduced by air filtration to a mean dosage indoors of 7 per cubic yard of air, or a reduction of 97 per cent. The maximum dosage of ragweed pollen on any one day was 20 per cubic yard of air, which was reached shortly after installation of the unit. After a few days' operation of the filter, no pollen was detected.

This unit, however, showed only a 53 per cent reduction of hormodendrum spores, with a mean dosage of these spores outdoors of 128 per cubic yard of air. The alternaria count was rather low in this area, 74 per cubic yard of air outdoors, with a reduction of 92 per cent indoors.

Location No. 2.—A small apartment was supplied filtered air by a unit and filter larger than that installed in location No. 1, but manufactured by the same company. Precautions were taken at this location prior to the ragweed season to reduce the pollen count as low as possible indoors by a thorough housecleaning to remove accumulated pollen from previous seasons and by the use of storm windows.

A nurse experienced seasonal ragweed hay fever. She was given a great deal of relief by air filtration shortly after coming in from outdoors and also upon awaking in the morning when ordinarily her symptoms would be most severe.

The mean dosage of ragweed pollen outdoors at this location was 195 per cubic yard of air, while indoors it was 7 per cubic yard of air. The maximum dosage at any time indoors was 16 per cubic yard of air, which was reached on just one day. The over-all reduction indoors during the height of the ragweed pollen season amounted to 97 per cent.

*Filter, described as an "air mat" filter consisting of six-ply mat material produced from spruce pulp and treated with a colorless, odorless oil, matting encased in a cardboard frame, two-inch convolutions with separators between to increase the surface area, filter size 17 by 9 by 2 inches.

The mean dosage of hormodendrum spores was 820 per cubic yard of air, with a reduction of 91 per cent by air filtration. The mean dosage of alternaria spores was 149 per cubic yard of air, with a reduction of 88 per cent by air filtration.

Additional readings taken in another apartment of the same size, but on the second floor of this building with no air filtration and no precautions taken from the standpoint of windows and doors, indicated a pollen and spore count approximately one-half that of the outdoor count.

Location No. 3.—A standard air conditioning unit* was installed in a five-room one-story home. Windows were kept tightly closed, but no additional precautions were taken except that prior to the pollen season there was a thorough housecleaning. This unit was operated during the first part of the summer on recirculation of indoor air, but in the latter portion on circulation of outdoor air through the unit. It was hoped that the reduction of ragweed pollen and spores by these two operating characteristics of air conditioning units could be determined under the conditions just described. In recirculation of indoor air there should be a higher natural leakage, as there is no positive pressure within the home. In circulation of outdoor air, although there may be less natural leakage because of some positive pressure, the efficiency would depend to a greater extent on the filter used by the unit and on the degree to which pollen and spores might be removed by the condensation of moisture on the cooling coils.

The owner of this residence has experienced hay fever due to a ragweed sensitivity during the past fifteen years and has had asthmatic symptoms, especially at night, during the past five years. Symptoms of hay fever subsided shortly after returning home in the evening and, although some asthmatic symptoms did occur at night, they were much milder compared with previous seasons.

With a mean dosage of ragweed pollen outdoors of 70 per cubic yard of air, by recirculation of indoor air this was decreased by 98 per cent. At no time did the ragweed pollen count indoors exceed 4 per cubic yard of air. A comparison of indoor and outdoor spore counts showed a reduction of 86 per cent for alternaria and 89 per cent for hormodendrum.

In circulation of outdoor air through this unit there was a reduction of 81 per cent for ragweed, 60 per cent for alternaria, and 77 per cent for hormodendrum. The ragweed pollen dosage indoors reached a maximum of 24 per cubic yard of air, which was the one night that asthmatic symptoms occurred of sufficient severity to interfere with sleep. The mean dosage of ragweed pollen indoors was 18 per cubic yard of air.

A comparison of the figures obtained using recirculation of indoor air with circulation of outside air indicates how much less effective these units are in the control of both ragweed pollen and spores by operation on "fresh air."

*Filter, a permanent filter constructed of coarse mesh screening into which is sprayed a special filter oil supplied with the unit. Filter size 20 by 9 by ½ inches.

Location No. 4.—A small room for gastroscopy in the hospital was air conditioned for comfort of personnel. No precautions as to pollen or spores were taken in this location. Again, in tests on this unit,* the air in this room was first recirculated followed by additional readings on circulation of outside air through the unit.

The mean ragweed pollen dosage indoors in recirculation of indoor air was 7 per cubic yard of air, with a total reduction of 94 per cent. These readings were taken during an outdoor ragweed pollen count of 116 per cubic yard of air. The mean hormodendrum count per cubic yard of air outdoors was 1,088; that of alternaria was 300, and the reduction by recirculation was 94 per cent for hormodendrum and 93 per cent for alternaria.

Although the outdoor count for both ragweed and spores was much lower during circulation of outdoor air, the reduction again was much less—86 per cent for ragweed pollen, 78 per cent for alternaria and 80 per cent for hormodendrum spores.

SUMMARY

A total of approximately 100 air samples was taken by a volumetric sampler at four widely separated locations to determine the efficiency of certain mechanical filters and air conditioners in the removal of ragweed pollen and hormodendrum and alternaria spores. All these air samples were taken during the height of the ragweed season and, because of the marked diurnal variation of ragweed pollen, all readings were taken between 11:00 A.M. and 1:00 P.M.

These volumetric readings indicate that mechanical filters are available which, by filtration, will reduce the incidence of ragweed pollen indoors to a mean dosage of 7 per cubic yard of air. The outdoor count was approximately 200 per cubic yard of air at the two locations studied. Although these filters are remarkably effective in the removal of air-borne ragweed pollen, they are much less so in the removal of air-borne hormodendrum and alternaria spores.

Similar air samples show that air conditioning units, when operated on recirculation of indoor air, will markedly lower the incidence of ragweed pollen indoors, comparing favorably in the results produced with mechanical filters. However, when the two units tested were operated on circulation of outside air, they were found to be much less effective in the removal of both ragweed pollen and fungus spores.

Complete dependence cannot be placed on either mechanical filters or air conditioning units for the control of air-borne hormodendrum and alternaria spores.

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BIOLOGIC EFFECTS OF MATERIALS DERIVED FROM STAPHYLOCOCCUS: FURTHER STUDIES

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PREVIOUS studies from this laboratory,[1] concerning the role of staphylococcus in bacterial hypersensitivity, have demonstrated that an extract of a pathogenic strain of staphylococcus possessed certain consistent toxic effects upon the isolated ileum of the guinea pig. In contrast, an extract from a nonpathogenic strain of staphylococcus, prepared in the same manner, was not toxic. This toxic activity was limited to one fraction, probably a nucleoprotein. Our extract[2] differs in preparation from those used for the most part, by earlier workers,[6] as it is derived from organisms rather than from culture filtrate.

In the present investigation we have confirmed and extended our previous observations to include comparative studies with other materials derived from staphylococcus.

MATERIALS AND METHODS

The materials used in the present study were derived from two major sources: from bacterial washed free of media and soluble products, on the one hand, and from culture filtrate free of bacteria on the other. The former will be referred to as "bacterial extract" and the latter as "culture filtrate."

Three bacterial extracts were studied. The first was derived from a strain of hemolytic *Staphylococcus aureus* (F-10), obtained from an antral washing of a patient (F) with atopic dermatitis, asthma, and chronic sinusitis. This strain is one of those used in our previous studies.[1, 2] The second bacterial extract was obtained from a Wood 46 strain of hemolytic *Staphylococcus aureus*, kindly supplied by Lederle Laboratories, and is the strain used in the production of Lederle staphylococcus toxoid. The third bacterial extract was obtained from a mixture of two strains each of *Staphylococcus aureus* (Cultures 1016 and 1035) and *albus* (Cultures 1309 and 1311), and was kindly prepared and supplied by Eli Lilly and Company, being identical with their commercially available undenatured bacterial antigen (U.B.A.), except that it contained no preservative.

The bacterial extracts F-10 and W-46 were prepared, as previously reported,[1, 2] from washed organisms ground in a ball mill for forty-eight to seventy-two hours, and then suspended in buffered saline and passed through a

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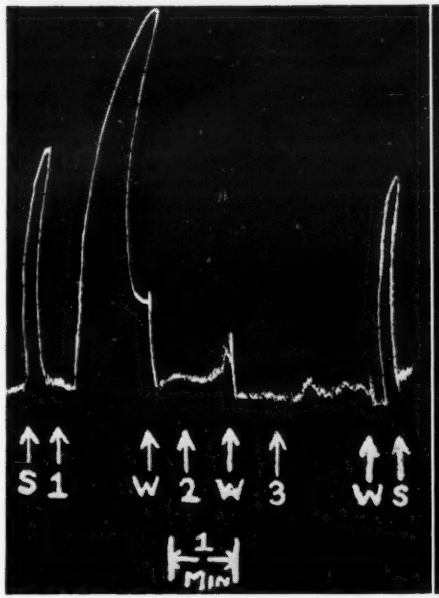

Fig. 1.

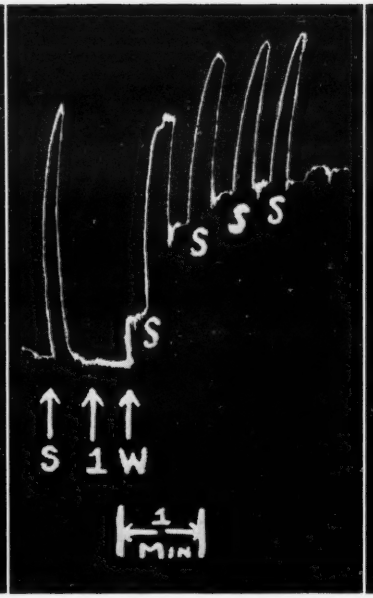

Fig. 2.

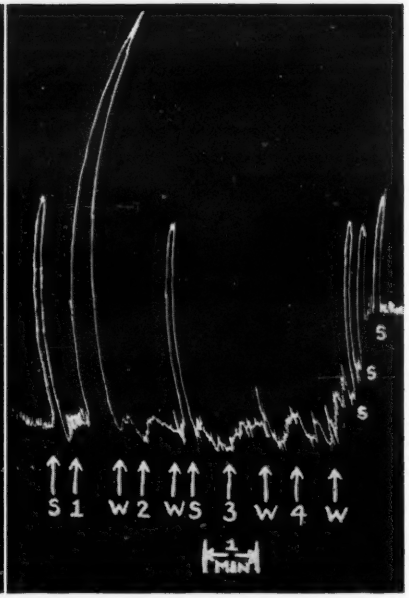

Fig. 3.

Fig. 1.—Effect of bacterial extract of pathogenic strain of staphylococcus (F-10) upon isolated suspended segment of ileum from a normal guinea pig. *S* indicates addition of 0.1 μg of standard histamine base followed by washing; *W* indicates three washes.

Arrow 1, 0.015 mg. P.N. extract (F-10) added to bath. Maximal contraction after fifteen to thirty seconds with relaxation in one minute.

Arrow 2, 0.03 mg. P.N. extract (F-10) added to bath. No contraction in one minute (tachyphylaxis).

Arrow 3, 0.13 mg. P.N. extract (F-10) (1951 batch) added to bath. No contraction in one minute. This demonstrates the cross tachyphylaxis between two separate batches of extract.

Fig. 2.—Effect of culture filtrate of pathogenic strain of staphylococcus (W-46) upon isolated suspended segment of ileum from normal guinea pig. *S* and *W*, same as Fig. 1.

Arrow 1, 0.18 mg. P.N. culture filtrate added to bath. No response in thirty seconds, but progressively increasing tonicity of segment after washing, unaffected by repeated additions of histamine standard and washes.

Fig. 3.—Effect of initial challenge with staphylococcus extract on subsequent challenge with culture filtrate upon isolated segment of normal guinea pig ileum and difference between extract and filtrate with respect to heat stability. *W* and *S*, same as Fig. 1.

Arrow 1, 0.015 mg. P.N. extract (F-10) heated at 100° C. for fifteen minutes. Typical contraction as in Fig. 1, demonstrating heat stability.

Arrow 2, 0.03 mg. P.N. extract (F-10) unheated. No reaction (tachyphylaxis).

Arrow 3, 0.35 mg. P.N. culture filtrate heated to 100° C. for fifteen minutes. No reaction, demonstrating heat lability.

Arrow 4, 0.35 mg. P.N. culture filtrate, unheated. Typical reactions as in Fig. 2.

Seitz filter. F-10 cultures were harvested at six to eight hours, in many instances, to coincide more closely with the optimum period of growth, and in other cases they were harvested at forty-eight hours, as in previous studies.[2] W-46 cultures were harvested at forty-eight hours. F-10 organisms were lyophilized before grinding, whereas W-46 organisms were not. The undenatured bacterial antigen (U.B.A.) was prepared[4] very similarly to the F-10 and W-46 bacterial extracts, with only minor differences. Like W-46 bacterial extract, it was not lyophilized.

Two culture filtrates, from F-10 and W-46 strains, respectively, were studied. These were obtained from forty-eight-hour cultures grown in an atmosphere of 80 per cent oxygen and 20 per cent carbon dioxide, the bacterial sludge being used for the preparation of the bacterial extract. The culture filtrates were dialyzed against running water for several hours to remove the histamine-like activity, which was regularly present.

TABLE I. EFFECT UPON INTACT ANIMALS OF MATERIALS DERIVED FROM PATHOGENIC STAPHYLOCOCCUS

ANIMAL AND ROUTE OF ADMINISTRATION	BACTERIAL EXTRACT				CULTURE FILTRATE		
	DOSE	F-10	W-46	U.B.A.	DOSE	F-10	W-46
Guinea Pigs 250 to 400 Gm. (I.V.)	0.10 mg. P.N.	0/2*					
	0.14 mg. P.N.	0/2					
	0.20 mg. P.N.			0/1			
	0.21 mg. P.N.	0/1					
	0.38 mg. P.N.	0/1†			0.35 mg. P.N.	0/1	1/3D‡
	0.40 mg. P.N.			0/3			
	0.50 mg. P.N.			0/1			
	0.63 mg. P.N.	2/2S†					
	0.71 mg. P.N.	0/1			0.70 mg. P.N.		0/1
	0.77 mg. P.N.	2/2S					
	0.81 mg. P.N.	0/2					
	1.10 mg. P.N.			0/2	1.05 mg. P.N.		2/2D
	1.28 mg. P.N.	0/1			1.38 mg. P.N.		1/1D
	1.60 mg. P.N.		0/3		1.42 mg. P.N.	1/1D	
	2.86 mg. P.N.	0/1			1.70 mg. P.N.	1/1D	0/3†
Rabbits (I.V.)	0.10 mg. P.N./Kg.		0/1		0.004 mg.P.N./Kg.		0/4
	0.12 mg. P.N./Kg.	0/1			0.04 mg. P.N./Kg.		6/6D
	0.16 mg. P.N./Kg.	0/1		0/1	0.07 mg. P.N./Kg.		2/2D
	0.20 mg. P.N./Kg.			0/1	0.14 mg. P.N./Kg.		3/3D
	0.31 mg. P.N./Kg.	0/1			0.49 mg. P.N./Kg.		1/1D
Kittens (I.P.)	0.18 mg. P.N.	0/3†			1.26 mg. P.N.		0/3†
	0.63 mg. P.N.			0/2			

S=Symptoms only.
D=Death.
*Number of positive reactions/total number of animals.
†Heated at 100° C. for fifteen minutes.
‡Old obliterative pleurisy and pulmonary atelectasis.

Arrow 2, 0.35 mg. P.N. culture filtrate heated to 100° C. for fifteen minutes. No reaction, demonstrating heat lability.
Arrow 4, 0.35 mg. P.N. culture filtrate, unheated. Typical reactions as in Fig. 2.

The bacterial extracts and culture filtrates were investigated with regard to their effects upon normal rabbits and guinea pigs (Table I) and upon isolated segments of guinea pig ileum suspended in a 5 ml. bath containing Tyrode solution maintained at 37° C. (Table II and Figs. 1 through 8). Similar studies were carried out on certain of these materials after treatment with 0.3 per cent Formalin for four to six weeks or heating at 100° C. for fifteen minutes. Form-

alinization produced no gross changes in the physical properties of the materials studied, but heating produced a white precipitate in all materials. In these cases studies were carried on with a total suspension, rather than with the clear supernate.

Twenty-two normal rabbits received intravenous injections of bacterial extracts and culture filtrates (Table I). Sixteen animals were given increasing doses of culture filtrate up to L.D. 100, and six were given bacterial extract in amounts up to several times the L.D. 100 of culture filtrate.

TABLE II. BIOLOGICAL EFFECTS OF MATERIALS DERIVED FROM STAPHYLOCOCCUS

	BACTERIAL EXTRACT							CULTURE FILTRATE				
	F-10			W-46		U.B.A.		F-10		W-46		
EFFECT	UNTREATED	HEATED AT 100° C.	FORMALINIZED	UNTREATED	HEATED AT 100° C.	UNTREATED	HEATED AT 100° C.	UNTREATED	HEATED AT 100° C.	UNTREATED	HEATED AT 100° C.	FORMALINIZED
Contraction of isolated guinea pig ileum (see illustrations and text)	+	+	0	+	+	+	+	+	0	+	0	0
Lethal effect (see Table I)	0	0		0		0		+		+	0	
Production of antilethal effect	0									+		+
Hemolytic effect	0					0				+	0	0
Production of antihemolysin	0									+		+
Dermonecrotoxic effect	0					0		+		+	0	0
Production of antidermonecrotoxin	0									+		+

Thirty-eight guinea pigs, weighing 250 to 400 grams, received intravenous injections of bacterial extracts and culture filtrates (Table I). Thirteen animals were given culture filtrate in doses up to L.D. 100, and twenty-five were given bacterial extracts in doses up to well in excess of the L.D. 100 of culture filtrate.

Tests on the isolated guinea pig ileum were made, as previously described,[2] by suspending one- to two-inch segments in a 5.0 ml. bath containing Tyrode solution, maintained at 37° C., and recording changes in length of the segments on a revolving smoked drum. Effects of the various materials were studied on ninety-nine segments of ileum from twenty-eight normal guinea pigs (Table II, and Figs. 1 through 8).

To test for the presence of enterotoxin,[5] five kittens received equal volumes of bacterial extract (F-10 and U.B.A.), and three received culture filtrate (W-46) intraperitoneally (Table I), after these materials had been heated for fifteen minutes at 100° C. Animals were then observed for one hour for vomiting, diarrhea, and shock.

Tests for hemolysin in bacterial extract (F-10 and U.B.A.) and in culture filtrate (W-46) were carried out on 1 per cent suspensions of normal rabbit red blood cells.[3]

Tests for dermonecrotoxin were performed by the intradermal injection in normal rabbits of bacterial extract or of culture filtrate and by observation for erythema and edema, with or without necrosis, after twenty-four to forty-eight hours.

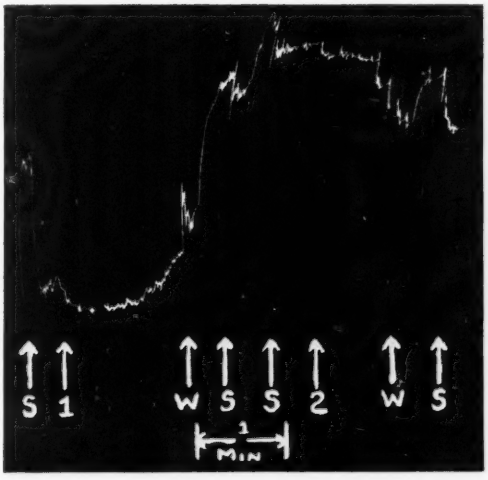

Fig. 4.—Effect of initial challenge with staphylococcus culture filtrate on subsequent challenge with extract upon isolated segment of normal guinea pig ileum (large dose of filtrate). *S* and *W*, same as Fig. 1.

Arrow 1, 0.18 mg. P.N. culture filtrate (W-46) added to bath. Gradually increasing tonicity to maximal, unaffected by repeated additions of histamine standard and washes.

Arrow 2, 0.015 mg. P.N. extract (F-10). No response. Note unresponsiveness to histamine standard (*S*) at conclusion of experiment.

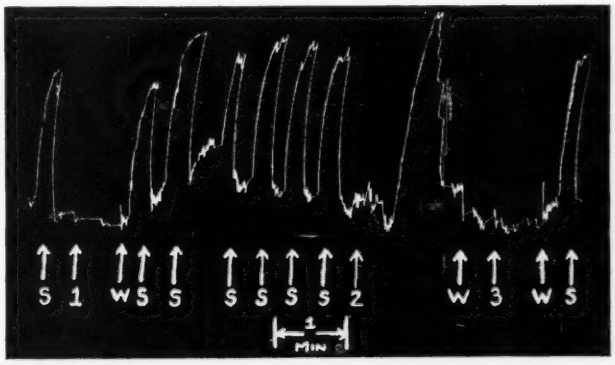

Fig. 5.—Same as Fig. 4, except using small dose of staphylococcus culture filtrate.

Arrow 1, 0.07 mg. P.N. staphylococcus culture filtrate (W-46) added to bath. Transient slight increase in tonicity with slight elevation of base line. Return to original level after several additons of histamine standard and washes.

Arrow 2, 0.015 mg. P.N. extract (F-10) added to bath. Typical contraction.

Arrow 3, 0.015 mg. P.N. extract (F-10) added to bath. No response (tachyphylaxis).

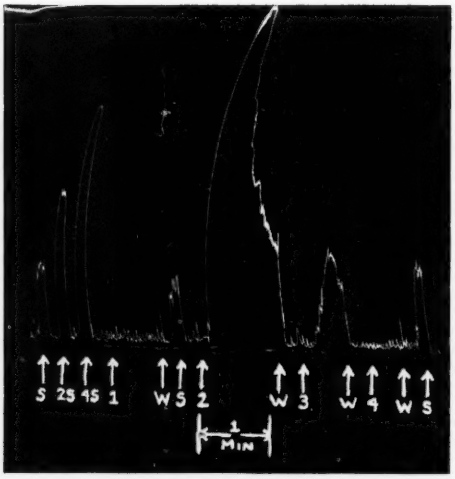

Fig. 6.—Effect of initial challenge with Formalin-treated staphylococcus extract on subsequent challenge with extract upon isolated ileum from normal guinea pig. *S* and *W* same as Fig. 1. *S, 2S,* and *4S* demonstrate quantitative response to histamine.

Arrow 1, 0.015 mg. P.N. Formalin-treated (dialyzed) staphylococcus extract (F-10) added to bath. No response.

Arrow 2, 0.015 mg. P.N. untreated extract (F-10). Typical maximal contraction.

Arrow 3, 0.03 mg. P.N. extract (F-10). Response much less than Arrow 1 (incomplete tachyphylaxis).

Arrow 4, 0.03 mg. P.N. extract (F-10). No response (tachyphylaxis).

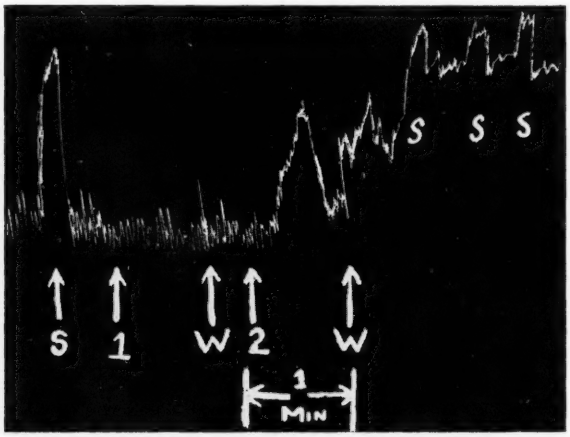

Fig. 7.—Effect of initial challenge with Formalin-treated staphylococcus culture filtrate (toxoid, Lederle) upon subsequent challenge with untreated filtrate. *S* and *W*, same as Fig. 1.

Arrow 1, 0.08 mg. P.N. toxoid added to bath. No response.

Arrow 2, 0.35 mg. P.N. untreated culture filtrate (W-46) added to bath. Typical response as seen in Fig. 2.

Tests for antilethal, antihemolytic, and antidermonecrotoxic activity were done on intact rabbits or sera from rabbits five to seven days after completion of a five-week course of two injections a week of the various materials. Most of the immunizing injections were given intravenously, a few intradermally (Table III).

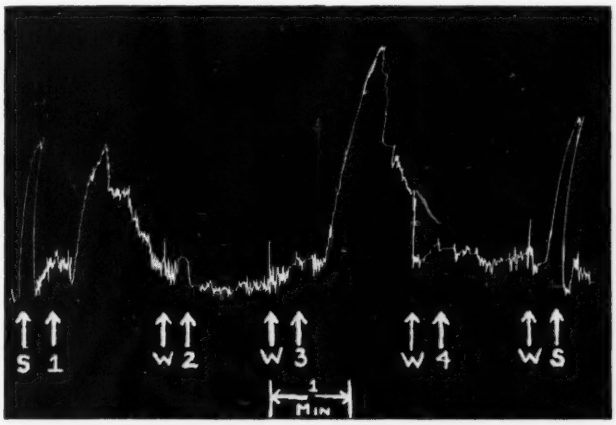

Fig. 8.—Effect of bacterial extract, U.B.A. (Lilly), upon isolated guinea pig ileum and upon subsequent challenge with extract (F-10). *S* and *W* as in Fig. 1.

Arrow 1, 0.22 P.N. mg. U.B.A. (dialyzed) added to bath. Moderate contraction in fifteen seconds similar to that of extract (Fig. 1).

Arrow 2, 0.22 P.N. mg. U.B.A. No response (tachyphylaxis).

Arrow 3, 0.015 mg. P.N. bacterial extract (F-10) added to bath. Maximal contraction as in Fig. 1.

Arrow 4, 0.015 mg. P.N. bacterial extract (F-10). No response (tachyphylaxis).

Antilethal activity was determined in rabbits, previously injected according to the schedule just described, by challenging them intravenously with doses of culture filtrate in excess of the L.D. 100 (Tables II and III).

TABLE III. TESTS FOR ANTILETHAL (AL), ANTIHEMOLYTIC (AH), AND ANTIDERMONECROTOXIC (AD) ACTIVITY IN RABBIT SERUM AND INTACT ANIMALS

MATERIAL	ANIMAL	TOTAL DOSE	SERUM		INTACT ANIMAL	
			AH (STANDARD UNITS)	AD	AD	AL
Bacterial Extract (F-10)	85	0.445 mg. P.N. (I.V.)	0			0
	76	0.520 mg. P.N. (I.V.)	0	0		0
	51	1.48 mg. P.N. (I.V.)	0			
Culture Filtrate (W-46)						
Untreated	82		0.8		0	+
	99	0.02 mg. P.N.				0
	100	(Intradermal)				0
	101					0
	84	0.318 mg. P.N. (I.V.)	0.1	+		
Formalinized (Toxoid)	78	0.049 mg. P.N. (I.V.)	0.1		+	+
	75	0.057 mg. P.N. (I.V.)	0.1		0	+
Pepsin digested	77	10.03 mg. P.N. (I.V.)	0.4	+	+	+
Normal Controls	68*		1.6			
	8 animals		all 0			
	8 animals					all 0

*At time of bleeding, observed to have purulent discharge from eyes and sores on feet.

Tests for antihemolysin were carried out (Tables II and III) following the method of Leonard and Holm,[3] in which the antihemolytic activity is determined against a test dose of hemolysin which, in turn, has been standardized against $\frac{1}{50}$ unit of standard antitoxin (National Institutes of Health). The antihemolysin is expressed, therefore, as standard units (Table III).

Tests for antidermonecrotoxin were carried out by a modification of the preceding test, using the same method, except that the end point is protection against erythema and edema following intradermal injection of the test dose of dermonecrotoxin.

RESULTS

These studies have shown, in general, that there are striking qualitative and quantitative differences in biologic effects of material derived from bacterial extract, on the one hand, and culture filtrate on the other (Tables I and II, Figs. 1 through 8).

Effect on Intact Animals.—Culture filtrate possesses lethal activity for rabbits and guinea pigs, which is not demonstrable with extract (Table I).

W-46 culture filtrate, 0.04 mg. or more of protein nitrogen (P.N.) per kilogram, killed all of twelve rabbits, whereas from 0.10 to 0.31 mg. P.N. per kilogram of extract (W-46, F-10, or U.B.A.) caused no reaction in six rabbits. Intravenous injection of a large lethal dose of culture filtrate into a rabbit after a latent period of about two minutes results in a reaction characterized by paralysis of the hind limbs, then total paralysis, collapse, and death in a few minutes. With smaller fatal doses around the M.L.D. 100, the reaction begins after a latent period and consists of shivering, staggering, gradual loss of function of the hind limbs, then total paralysis, collapse, and death occurring from twenty minutes to three hours after the injection, the latent period extending up to fifteen to thirty minutes before death. Gross autopsy findings reveal the heart in ventricular standstill or twitching feebly. There is no evidence of the pulmonary arterial obstruction characteristic of anaphylactoid reactions in rabbits.

F-10 and W-46 culture filtrates given intravenously to five guinea pigs in doses of over 1.0 mg. of protein nitrogen killed all the animals, while bacterial extract in doses of more than twice the L.D. 100 of culture filtrate failed to kill guinea pigs. The fatal reactions in the guinea pig, as in the rabbit, are characterized mainly by paralysis and collapse. The animal, after a latent period of a few minutes, develops flaccid paralysis, first of the hind quarters, then generalized, with collapse and death in five to ten minutes from the time of injection. Pallor is sometimes apparent, but not cyanosis, and no respiratory distress is seen until paralysis is generalized. Convulsive movements occasionally precede death. Gross autopsy examinations are unremarkable except for the finding of the heart in ventricular fibrillation or in standstill with fibrillary twitching. There is no evidence of the pulmonary emphysema characteristic of anaphylactoid reactions in guinea pigs.

Lethal activity of the culture filtrate, as tested in guinea pigs, is destroyed by heat treatment (Table II).

Enterotoxin, as tested by intraperitoneal injection into kittens, is undetectable in heated bacterial extract or culture filtrate (Table I), since no untoward reactions were observed following these injections.

Injection into rabbits of untreated culture filtrate, as well as culture filtrate inactivated by Formalinization, resulted in production of antilethal antibody, as evidenced by subsequent protection of the rabbit against lethal doses of culture filtrate (Tables II and III).

Similarly, while both the hemolytic and dermonecrotoxic activities of the culture filtrate were destroyed by heat or Formalinization, injection of Formalin-treated material into rabbits produced circulating anti-hemolysin and antidermonecrotoxins, respectively (Tables II and III).

Bacterial extracts were inactive as regards all these types of activity (Tables II and III).

Effect on Isolated Guinea Pig Ileum.—While both culture filtrate and bacterial extract produce effects on the guinea pig ileum (Table II), these effects are strikingly different in many ways (Figs. 1 and 2). Initial challenge with extract (Fig. 1) causes maximal contraction of the segment in fifteen to thirty seconds, with relaxation in about one minute. Subsequent challenge with extract results in slight response or none, demonstrating tachyphylaxis. In contrast, addition to the bath of culture filtrate (Fig. 2) causes a gradually increasing tonicity with a rising level of the base line. Repeated additions of histamine, followed by washes, have no effect upon this contracted state, which persists indefinitely when a sufficient dose of filtrate is used (Figs. 2 and 4). Smaller doses (Fig. 5, arrow 1) have milder and more transient effects. Tachyphylaxis does not follow administration of culture filtrate if the doses used are suitable for this study. Large doses of filtrate render the gut totally unresponsive (Fig. 4), whereas doses of extract in excess of that producing maximum effect cause no such unresponsiveness.

Cross tachyphylaxis between extract and culture filtrate (Figs. 3, 4, and 5) does not occur, although if large doses of filtrate are used in the initial challenge the reaction to subsequent challenge with extract may be absent because of the total unresponsiveness of the gut (Fig. 4).

While Formalin destroys the activity of both culture filtrate and bacterial extract (Figs. 6 and 7), heating at 100° C. for fifteen minutes destroys the activity only of the filtrate, that of the extract being unaffected (Fig. 3, arrows 1 and 3).

Formalin-treated bacterial extract and culture filtrate confer no protective action upon subsequent challenge with extract and filtrate, respectively (Figs. 6 and 7).

Undenatured bacterial antigen (U.B.A.) has a similar effect upon the isolated guinea pig ileum, although weaker and less consistent than the F-10 and W-46 bacterial extracts (Fig. 8), requiring approximately fifteen times the amount of protein nitrogen to give a response of the isolated ileum comparable to that following the latter materials. Subsequent challenge with U.B.A. results in no reaction, demonstrating tachyphylaxis, although the gut still is responsive to F-10 bacterial extract (Fig. 8). Whether this lack of cross tachy-

phylaxis is due to qualitative differences between toxins from different strains of staphylococcus or whether it is only a matter of quantitative differences is still under investigation. The latter explanation is thought to be the most likely, since U.B.A. has a weaker and less consistent response and because initial challenge with our bacterial extract regularly causes cross tachyphylaxis with subsequent challenge with U.B.A.

DISCUSSION

The clinical immunologist has available for use a variety of materials derived from staphylococcus. These include suspensions of washed, killed, whole, or ground organisms, free of culture filtrate, solutions of Formalin-treated culture filtrate, free of organisms, and combinations of materials derived from both organisms and filtrate. While the present work has been concerned with studies of certain biologic activities of a cellular extract as compared with a cell-free culture filtrate of pathogenic staphylococcus, with emphasis on toxic properties, it is probable that there are many other nontoxic proteins within the cellular structure which are absent, or nearly so, from culture filtrate. We have shown previously[2] that the toxic activity in the bacterial extract from a pathogenic strain (F-10) of staphylococcus is present in a protein fraction—probably a nucleoprotein—and that this same fraction from a non-pathogenic strain (F-7) of staphylococcus was nontoxic, yet was similar by analysis for nitrogen, phosphorus, carbohydrate, and total purines and was capable of sensitizing and causing anaphylaxis in guinea pigs, as were other nontoxic fractions from both the pathogenic and nonpathogenic strains.

The material derived from bacteria in a focus of staphylococcal infection in body tissue undoubtedly is made up of intracellular and extracellular, toxic and nontoxic proteins. It is reasonable to assume that immune or hyperimmune responses may occur to many or all of these proteins. Thus, it is felt that the use of either cellular extracts or culture filtrate alone in laboratory studies or clinical use may be immunologically incomplete and that some combination of both should be more suitable.

SUMMARY AND CONCLUSION

Further studies with bacterial extracts of pathogenic staphylococcus washed free of culture filtrate have shown that the effects of these extracts upon the isolated ileum of the guinea pig, as well as in the intact guinea pig and rabbit, are strikingly different from those of bacteria-free staphylococcus culture filtrate similar to that used, for the most part, by workers interested in this field, even when the culture filtrate and bacterial extract are derived from the same organism at the same time.

Culture filtrate is lethal for intact rabbits and guinea pigs, whereas bacterial extract is not. Culture filtrate also possesses hemolytic and dermonecrotoxic activity, not found in bacterial extract.

Injection of untreated or Formalinized culture filtrate into rabbits results in the development of immunity against the lethal effects and of antihemolysin

and antidermonecrotoxin as well. Injection of bacterial extract within the limits of our experimental methods results in no such effects. Further studies, using different immunizing techniques, are in progress.

Both bacterial extract and culture filtrate have toxic effects upon the isolated guinea pig ileum, but these differ strikingly from each other. In addition, the toxic effect of bacterial extract is heat stabile, while that of culture filtrate is heat labile.

These findings should serve to emphasize the importance of proteins, toxic or not, which are present within the staphylococcus and which may play an important role in infection and hypersensitivity associated with this organism. Studies with culture filtrates alone, therefore, may overlook important toxic and allergenic factors within the cellular structure of these bacteria.

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ACETIC ACID SENSITIVITY AS A CAUSE OF COLD URTICARIA

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THE subject of cold urticaria has been well documented since Blachez[1] first reported the condition in 1872 after Bourdon[2] had initially recognized it in 1866. Since then, many others have written on the subject, among them Lewis and Grant,[3] Horton and Brown,[4, 5] Duke,[6] Bray,[7] Urbach,[8-10] Walzer,[11] Notier and Roth,[12] Sherman and Seebohm,[13] Rajka and Asboth,[14] Von Gerd,[15] Illig,[16] Herlitz,[17] and Sheldon, Mathews, and Lovell.[18] These authors have either presented cases describing the syndrome or discussed the probable immunologic mechanisms responsible for it, but no one has presented a case of cold urticaria which has been proved to be caused by a specific antigenic factor. To date, the cure of true allergic cold urticaria by means of allergic management has not been reported. It is the purpose of this discussion to describe a patient with a prolonged history of severe cold urticaria whose symptoms were related to the ingestion of acetic acid and were proved to be caused by a specific sensitivity to this acid. Since this patient had a remarkable clinical course and obtained a complete remission following allergic management, it seemed worth while to present the study of his case, especially with the thought in mind that new fields of investigation regarding causative mechanisms of physical allergies might be opened.

CASE PRESENTATION

P. A., a 35-year-old single male baker, was first seen on Jan. 26, 1954. He gave a four-year history of recurrent bouts of hives on the uncovered parts of the body following exposure to cold. The major areas involved were the head, face, ears, feet, and hands. He also complained of swelling in his throat following the ingestion of ice cream or very cold drinks. During recent years the hives became progressively worse, flaring up each fall at the onset of cold weather. The patient stated that he never had hives after swimming during the summer months. Antihistamines afforded him only temporary relief, and it was often necessary for him to remain in a warm room for over an hour before the swelling subsided. No symptoms suggesting dermographism were elicited. Hives had occurred intermittently all his life, and as a youngster he used to take Adrenalin under the tongue for relief of acute attacks. During the patient's childhood, citrus fruits, tomatoes, and certain brands of butter seemed to precipitate bouts of hives. He stated that he now ate tomatoes without difficulty, but still avoided citrus fruits. The patient had been a baker for the past ten years. He had lived at home with his parents in the lower half of a two-story house, where he had not been exposed to animal dander and had never followed dust precautions. Cottonseed flour was not used in the bakery, and no association between his symptoms and his work was noted. He used scented aftershave lotions and powders, and was exposed to average quantities of insecticides when the bakery was sprayed once a month by an exterminating service. The diet contained rather sizable quantities of chocolate, nuts, and spices, but the patient admitted a strong aversion to milk. He drank moderate amounts of beer and smoked six or seven cigars a day. Family history was completely negative for allergy as far as could be ascertained. Operations included a hemorrhoidectomy and a tonsillectomy.

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Complete physical examination was essentially negative except for an injected nasal mucous membrane and marked cloudiness on transillumination of the frontal and antral sinuses. Chest fluoroscopy, urinalysis, blood sugar, sedimentation rate, and Wassermann all gave normal results. The blood count showed a 5 per cent eosinophilia.

Scratch tests for dermographism were negative. When an ice cube was applied to his skin for three to five minutes, an urticarial lesion quickly formed in the exposed area. Blood was drawn and stored at refrigerator temperature for five weeks. Ice cube tests on sites in normal skin passively sensitized with the five-week-old serum were negative. The patient was seen approximately twice a week for further observation, and at each visit quickly showed a hive wherever an ice cube was placed on his skin.

Since patients with a physical allergy to cold usually get severe urticaria following swimming, the fact that our patient did not respond similarly was most puzzling. After exhaustive questioning, the patient finally remembered that he started to ingest very large quantities of sauerkraut at approximately the same time that his urticaria began four years previously. He was on a reducing diet and ate the sauerkraut because of its low caloric value. He remembered that he did not eat sauerkraut during the summer months, as it was unobtainable. He was therefore told to avoid sauerkraut and was observed for a change in his clinical condition. The following week the patient noticed a very definite diminution in his response to cold. When the ice cube was removed from the skin at this time, the hive did not form as quickly as had been previously observed, taking twenty to twenty-five minutes instead of three to five minutes.

During the next month, after institution of his elimination diet, he was quite comfortable but continued to keep a food diary. He noted that the ingestion of dill pickles, followed within an hour by a cold beverage, precipitated an edematous response in the mucous membrane of the hypopharynx. It was during a warm period in March that this reaction occurred. Since acetic acid is one of the main ingredients common to both sauerkraut and dill pickles, it was decided to investigate the possibility that sensitivity to this chemical compound was causing his allergic condition. With this in mind, the patient was advised to abstain from all foods which might contain acetic acid. He continued to be asymptomatic and, for the first time in four years, observed no hives following exposure to cold.

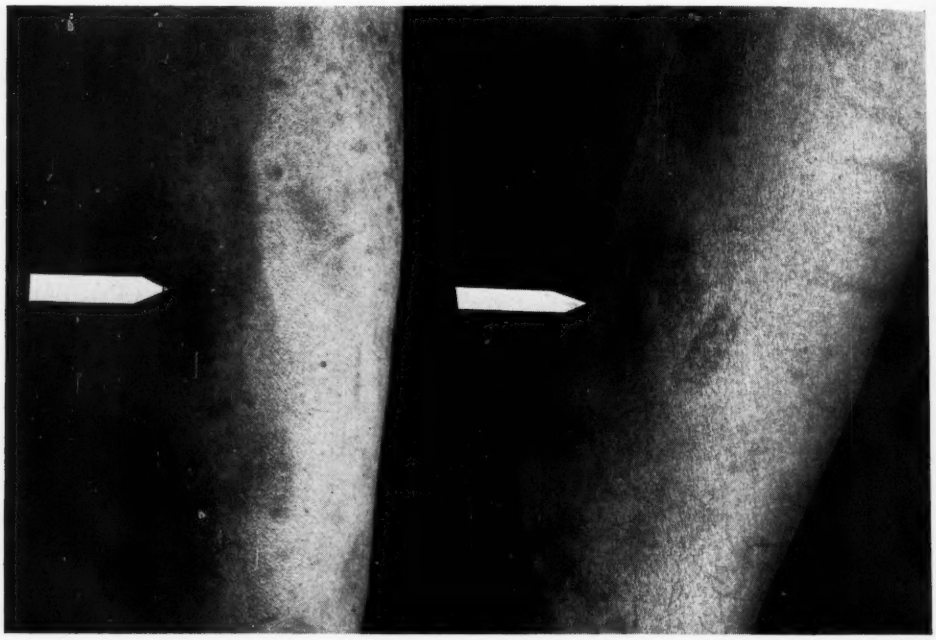
The sauerkraut was made from cranberries, cabbage, and salt, while the pickles contained vinegar, spices, thyme, dill, garlic, and sodium benzoate. On infrequent occasions when he had eaten plain cabbage or cranberries no unusual reactions had occurred. After obvious improvement had taken place during the first six weeks of the elimination diet, he again noted mild attacks of urticaria on exposure to cold shortly after adding sauerkraut or dill pickles to his diet. As soon as he avoided these foods, the hives subsided.

In order to eliminate the possibility that psychogenic factors may have influenced the results, special ingestion tests were performed. A thick carbohydrate placebo mixture with a strawberry-peppermint flavor was prepared with lemon juice added in an attempt to simulate an acid flavor. Because of the warm weather at this time, the only way the reaction could be reproduced was to have the patient consume some chilled material following ingestion of the test mixture. He was given about 15 ml. of the placebo mixture on two different occasions, following which he immediately ate some ice cream and noted no unusual reaction. He was then given 15 ml. of the placebo mixture with the addition of about 2 ml. of 2 per cent acetic acid. He again ate some ice cream and within fifteen minutes felt his throat begin to swell. The acetic acid had been obtained from ordinary vinegar. This 2 per cent solution was then diluted 1:1,000, 1:100, and 1:10 for skin testing purposes. Direct skin testing with these dilutions produced no greater reaction in the patient than was obtained on testing control subjects. It was observed that, in spite of his previous supposed sensitivity to citrus fruits, he could now ingest lemon juice, such as was used in the placebo mixture, without any untoward effects, indicating that probably he was no longer sensitive to lemon or other citrus fruits.

After a six-month lapse in observation, the patient has recently been restudied. He had been following the elimination diet very carefully. The ice cube test was now completely

negative, even after application to the skin of the forearm for a period of fifteen minutes. He was able to take pans out of a freezer at 10° F. in the bakery with his bare hands.

Because of the patient's previous history suggesting a possible sensitivity to citric acid and the fact that both lactic acid and acetic acid are found in large quantities in sauerkraut, it was decided to skin test him with chemically pure acetic, lactic, and citric acids. After no signs of cold urticaria had appeared for many months, skin tests on Dec. 7, 1954, with these .1 per cent chemically pure acids were negative. Tests done with .01 per cent acid solutions, followed by application of an ice cube over the skin test area for fifteen minutes, were also negative. When the intradermal tests were repeated, however, with .1 per cent acids, followed by application of an ice cube to the test sites for fifteen minutes, a definite urticarial response was obtained over the acetic acid site which gradually swelled into a wheal larger than a half dollar (4 cm. in diameter) during the next half-hour. No similar reaction was observed over the lactic acid or citric acid sites.



A. *B.*

Fig. 1.—*A*, Skin test with .1 per cent chemically pure lactic acid twenty-five minutes after ice cube was placed over test site. *B*, Skin test with .1 per cent chemically pure acetic acid twenty-five minutes after ice cube was placed over test site.

The same reaction was repeated on three separate occasions within a period of one week and each time, after removal of the ice cube from the acetic acid test site, the wheals began to form within five minutes and reached their maximum size five to ten minutes later (twenty to twenty-five minutes following the original contact of the ice with the acetic acid test site). Application of ice to the skin concomitantly for fifteen minutes in an area not previously skin tested, or in an area where a skin test with a .1 per cent lactic acid test had been performed, produced no urticarial response in either site (Fig. 1). The patient further stated that the lesion produced in the acetic acid test site definitely itched and felt like a typical hive. Placing an ice cube for fifteen minutes over a skin test with .1 per cent chemically pure acetic acid on several control subjects (nonallergic persons) gave completely negative reactions. Unfortunately, during the preliminary passive transfer studies of the

patient's serum, a skin test with acetic acid followed by an ice cube over the test site was not done. When this procedure was eventually carried out on the original serum after it had been refrigerated for ten months the test was negative.

Six weeks after the positive direct skin tests had been obtained, serum was withdrawn for additional passive transfer studies. Nonallergic persons were then sensitized with four-day-old filtered serum and were tested by placing an ice cube over an area previously injected with .1 per cent chemically pure acetic acid. No positive reactions were obtained. A few days later nonallergic subjects were sensitized with unfiltered serum four hours after it had been withdrawn. Skin testing of these sites with acetic acid plus an ice cube over the test site produced no positive responses, regardless of whether the tests were performed immediately or twenty-four hours following sensitization. Direct skin testing of the patient at this time with acetic acid in the presence of cold also produced a negative reaction. (See Table I for summation of testing results.)

TABLE I. SUMMATION OF TESTING RESULTS

DIRECT TESTING			INDIRECT TESTING		
DATE	TESTING MATERIAL	RESULTS	DATE	TESTING MATERIAL	RESULTS
3/20/54	Acetic acid .01%	Negative	2/ 1/54	Serum drawn	
3/20/54	Acetic acid .1%	Negative	3/ 8/54	Ice cube test on 2/1/54 serum	Negative
3/20/54	Ice cube test	Positive	12/ 7/54	.1% C.P.A.A. and ice cube over test site on 2/1/54 serum	Negative
12/ 7/54	.1% C.P.A.A.*	Negative			
12/ 7/54	.1% C.P.A.A. and ice cube over test site	Positive	1/14/55	Serum drawn	
12/ 7/54	Plain ice cube test	Negative	1/18/55	.1% C.P.A.A. and ice cube over test site on 1/14/55 serum	Negative
12/ 7/54	.1% C.P.L.A.†	Negative			
12/ 7/54	.1% C.P.L.A. and ice cube over test site	Negative	1/24/55	Serum drawn	
12/ 7/54	.1% C.P.C.A.‡	Negative	1/24/55	Tests performed on unfiltered serum with .1% C.P.A.A. and ice cube over test site 4 hours following withdrawal on 1/24/55	Negative
12/ 7/54	.1% C.P.C.A. and ice cube over test site	Negative			
12/ 9/54	.1% C.P.A.A. and ice cube over test site	Positive	1/25/55	Tests performed on unfiltered serum with .1% C.P.A.A. and ice cube over test site 24 hours following withdrawal on 1/24/55	Negative
12/ 9/54	.1% C.P.L.A. and ice cube over test site	Negative			
12/12/54	.1% C.P.A.A. and ice cube over test site	Positive			
12/12/54	.1% C.P.L.A. and ice cube over test site	Negative			
1/24/55	Plain ice cube test	Negative			
	.1% C.P.A.A. and ice cube over test site	Negative			

*C.P.A.A. chemically pure acetic acid.
†C.P.L.A. chemically pure lactic acid.
‡C.P.C.A. chemically pure citric acid.

He was then instructed to eat foods made with acetic acid to see if the hives would recur. During the next three months, he continued to ingest sauerkraut frequently without developing hives or any symptoms suggesting a mucous membrane hypersensitivity to cold.

The fact that we were not able to passively transfer the patient's reaction to acetic acid in the presence of cold means that if reagins were present they probably were produced for only a short time and that, if the studies could have been done at exactly the same time the direct reactions were obtained, reagins might have been demonstrated. Knowing that the reagenic sensitivity, which does occasionally occur in drug sensitivities, is usually of a transient nature, it is not surprising that our patient may have had a similar response.

The only previously recorded case of a patient who was thought to have cold urticaria from a specific chemical sensitivity was described by Herlitz.[17] He discussed a 15-month-old girl who appeared to have urticarial-like lesions on the exposed parts of her body after being out in cold weather. She was being treated at the time with Tulo tablets (throat lozenges containing menthol). The skin eruption had been present for only a few days and appeared to be directly related to the ingestion of these tablets, since the reaction stopped shortly after she was taken off the lozenges and reappeared following reinstitution of this medication. A confusing aspect of the case report, however, was the fact that when the patient was again given the lozenges, her skin lesions recurred following exposure to cold weather, but the ice cube test was negative. It is difficult to understand how one with active cold urticaria can have a negative ice cube test, especially when it was previously positive.

Abramson[19] reported a case of nonallergic cold urticaria which responded well to psychiatric therapy. There was no evidence in the patient's personal or family history to indicate that it was an allergic problem.

Urbach, Linneweh, and Greenberg[20] described a patient who developed urticaria after the ingestion of pork sausage which contained large amounts of pork blood. Following repeated exposures to the material, he eventually developed cold urticaria, as the lesions would only occur on areas just previously bathed with cold water. When given plain pork sausage which did not contain any pork blood, the reaction to cold did not take place. Since the patient signed out of the hospital prematurely, no further investigative studies could be conducted to determine what factor in the pork blood was responsible for the reaction.

Two additional cases of cold urticaria which responded well to treatment have also been reported by Urbach.[8, 9] One was a patient with a localized tooth infection whose urticaria cleared following removal of the tooth. The other was a 25-year-old woman with an intermittent twelve-year history of cold urticaria following the onset of her menses at age 13. The only periods free of urticaria during this time were while she was pregnant. The pelvic findings were normal, and the urticaria ceased after she received injections of posterior pituitary hormone combined with x-ray therapy to the pituitary gland.

Because of the belief that acetic acid is a normal constituent of body metabolism, it may be questioned whether hypersensitivity to acetic acid can occur. Personal discussion of this point with Dr. W. W. Westerfeld,[21] professor of biological chemistry at New York State College of Medicine, Syracuse, New York, has revealed the fact that currently it is believed that acetic acid is not a normal intermediate in body metabolism. As early as 1933, Himwich and his group[22] observed that no free acetic acid could be found in the blood following the metabolism of ethyl alcohol. Later, in 1939, Friedemann and Kmieciak[23] confirmed the work of Himwich and his associates,[22] adding further evidence to show that free acetic acid is not a normal breakdown product of body metabolism. In so far as is known, acetic acid is not present in the free state in any measurable quantity but is present in combination with a coenzyme,

CoA, as acetyl CoA. Because free acetic acid is not a normal product of body metabolism, it is possible that hypersensitivity to this compound could occur in human beings.

It would appear that the patient discussed in this article had a specific chemical sensitivity which produced the cold urticaria and that, following the elimination of this chemical from his diet, the allergic activity quickly subsided. The findings suggest that, in the presence of cold, either (1) the acetic acid acted as a hapten to form a complex antigen responsible for the allergic reaction or (2) the cold acted as a more suitable medium to permit the acetic acid hypersensitivity to take place. In this respect, the cold could have been a catalyst in the allergic reaction.

We believe that these findings are also significant because a new technique of testing for drug sensitivity may have been demonstrated. This type of testing may be especially valuable in the study of patients with physical allergies due to chemical sensitivities.

CONCLUSION

1. The case history has been presented of a patient who has apparently been cured of recurrent cold urticaria and mucous membrane hypersensitivity to cold of four years duration by avoidance of foods containing acetic acid.

2. After diet elimination, ingestion of a placebo mixture followed by ice cream produced no mucous membrane response. When a minute quantity of acetic acid was added to this mixture and the ingestion was followed by ice cream, pharyngeal edema quickly developed.

3. Direct skin testing with .1 per cent chemically pure acetic acid was negative, but when an ice cube was immediately placed over the test site for fifteen minutes an urticarial reaction resulted. Ice cube tests performed concomitantly over untested skin or over an area tested with .1 per cent chemically pure lactic or citric acid were negative. Similar testing with .1 per cent chemically pure acetic acid plus an ice cube over the test site in normal control subjects gave negative reactions.

4. A new technique for skin testing for drug sensitivities in patients suffering from physical allergies has been suggested.

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CLINICAL, CHEMICAL, AND PHARMACOLOGIC RELATIONSHIPS OF ANTIHISTAMINES

A Study of a New Synthetic Antihistaminic, Carbinoxamine Maleate (Clistin Maleate*)

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CORRELATION of chemical structure with the clinical and pharmacologic activity of antihistaminic drugs should lead to more useful therapeutic agents. This approach has been applied in many fields of chemotherapy and has led to new and better sulfonamides, sympathicomimetics, and arsenicals, to name only a few examples.

Our interest in this approach to the investigation of antihistaminic agents led us to initiate a clinical study with a new synthetic antihistaminic, carbinoxamine maleate (Clistin maleate).* The availability of this drug provided an unusual opportunity to correlate pharmacologic activity with chemical structure, since the chemical configuration of Clistin includes components which are comparable to those in several distinct classes of antihistaminic agents.

This report outlines the chemical relationships of Clistin to other well-known antihistaminic drugs and also correlates these chemical aspects with the pharmacologic effects produced by this new drug as compared with other antihistaminic agents. Clinical results obtained with the use of Clistin are also described.

The clinical studies summarized here were carried out during the fall, winter, and spring of 1953 in 200 patients at the National Institute of Allergic Diseases. Ninety-four of these patients were followed for a sufficient period of time to permit a satisfactory evaluation of the therapeutic effectiveness of Clistin and the incidence and type of side effects. The majority of the patients treated with Clistin had perennial allergic rhinitis, alone or complicated by bronchial asthma or asthmatic bronchitis. This fact may have provided an unusually severe test of this new drug, since perennial allergic rhinitis generally responds less dramatically to treatment with antihistaminics than is the case with seasonal allergic rhinopathy.

CHEMICAL RELATIONSHIPS BETWEEN CLISTIN AND OTHER ANTIHISTAMINICS

Clistin is the maleate salt of 2-[p-chloro-α-(2-dimethylaminoethoxy)benzyl] pyridine and has the following chemical structure:

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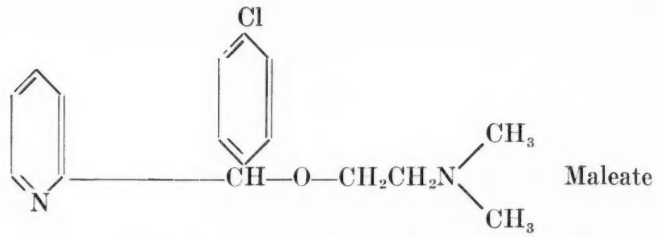
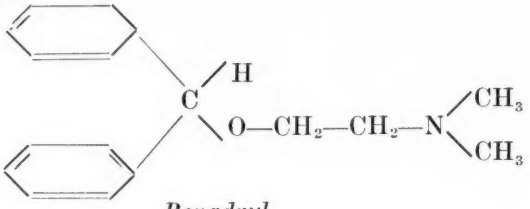
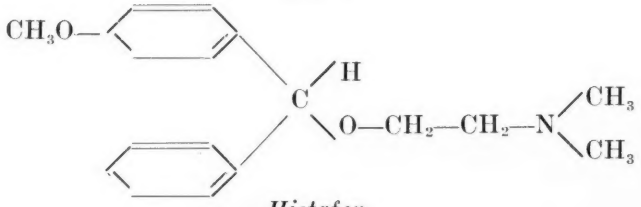


This chemical compound is a white crystalline material which is water soluble and stable to light and heat under ordinary conditions. Two per cent solutions have a slightly bitter taste but do not irritate the mucous membranes.

On analyzing the chemical structure of Clistin, it is seen to have another linkage comparable to that of diphenhydramine (Benadryl) or that of Histafen.*

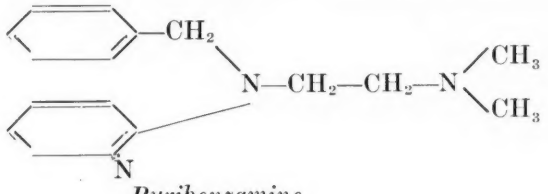


Benadryl

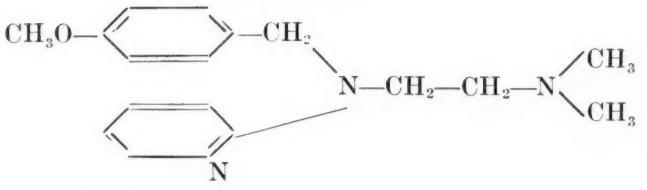


Histafen

Both Benadryl and Histafen have a benzhydryl nucleus, whereas Clistin has a benzene and a pyridine nucleus such as is present in tripelennamine (Pyribenzamine), pyrilamine (Neo-Antergan) and doxylamine (Decapryn).

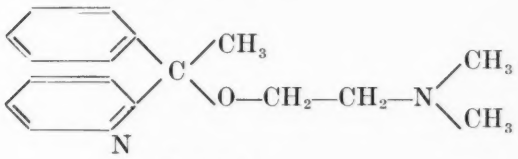


Pyribenzamine



*Lambelin, M.: Histafen, Bruxelles-méd. 28: 2282, 1948.

Neo-Antergan



Decapryn

Finally, Clistin has a chlorine atom added to the benzene nucleus, a characteristic which relates this new drug to 2[(p-chlorobenzyl)-(β-dimethylaminoethyl)amino]pyridine (Synopen*). It should be noted that this halogenation augments, in general, the antihistaminic action, as has been shown with various preparations, among which we cite pairs constituted by:

Pyribenzamine (nonhalogenated)	-	Synopen (halogenated)
	and	
Trimeton (nonhalogenated)	-	Chlor-Trimeton (halogenated).

This potentiation is also produced when halogenation is carried out on the heterocyclic nucleus, as in the case of methapyrilene (Histadyl, Thenylene), whose chlorinated derivative is Chlorothen or Tagathen. An identical increment in antihistaminic activity also occurs with brominated derivatives, as has been shown in the comparison of Histadyl and its brominated compound, Bromothen.

It may be said that Clistin is a new antihistaminic that has components of its chemical structure which are comparable to typical components of several other distinct classes of antihistaminics as represented by the other linkage of diphenhydramine (Benadryl), the benzene and pyridine nucleus of tripelennamine (Pyribenzamine), and the chlorine atom of chlorprophenpyridamine (Chlor-Trimeton).

TABLE I. ORAL PROTECTION OF GUINEA PIGS

COMPOUND	MEDIAN ORAL DOSE TO PROTECT 75 PER CENT OF ANIMALS GIVEN A CERTAIN LETHAL DOSE (1.0 MG. PER KG.) OF HISTAMINE DIHYDROCHLORIDE INTRAVENOUSLY ONE HOUR LATER (MG. PER KG.)	MEDIAN ORAL DOSE TO PRODUCE DEATH IN 50 PER CENT OF ANIMALS (MG. PER KG.)	RATIO $\frac{LD}{AH}$ (MG. PER KG.)
Clistin maleate	0.15	411	2740
Chlorprophenpyridamine maleate	0.15	210	1400
Prophenpyridamine maleate	1.75	270	154
Diphenhydramine hydrochloride	3.6	280	78
Tripelennamine hydrochloride	3.1	150	48

*Synopen (Avapena), Geigy Laboratory, Switzerland.

PHARMACOLOGIC ACTIVITY OF CLISTIN COMPARED WITH OTHER ANTIHISTAMINES

Numerous comparative studies have been carried out by Marsh[2, 3] to determine the activity and toxicity of Clistin as compared with other antihistaminics in various experimental animals. Typical of the results obtained are the data in Table I, which show that Clistin has a very wide ratio between the median effective dose (0.15 mg. per kilogram) and the median lethal dose (411 mg. per kilogram). This ratio, indicative of greater antihistaminic potency as well as of greater safety, was found to be from two to fifty times greater than the same ratio for the other drugs tested.

CLINICAL RESULTS

The ninety-four patients utilized to evaluate the clinical usefulness of Clistin had the following types of allergic disorders:

1. Perennial allergic rhinitis	68
2. Perennial allergic rhinitis with bronchial asthma or asthmatic bronchitis	12
3. Bronchial asthma	3
4. Pruritus or urticaria	9
5. Allergic conjunctivitis	1
6. Seasonal allergic rhinitis	1
Total	94

The age range was from 6 to 75 years, and sixty-eight of the patients were females. The weight range was from 18 to 91 kg. (40 to 200 pounds).

Dosage.—The dosage of Clistin was regulated according to weight, age, and sex.

Daily dosage of up to 24 mg. was usually administered in divided doses at intervals of two to eight hours. In general, therapy was initiated with 4 to 16 mg. daily, and the patients were advised to continue treatment if symptoms persisted or to decrease the dose when either appreciable improvement or undesirable side effects appeared. Sixty-three of the patients received specific hyposensitization in conjunction with this treatment, whereas the remaining thirty-one patients were treated with Clistin alone.

The clinical results were designated as *excellent* when the symptoms disappeared and no toxic manifestations developed, *very good* when the improvement was considerable and only mild side effects appeared, *good* when the relief was significant but definite subjective signs of intolerance existed, *fair* when the relief was scant or the side effects were significant, and *poor* when there was no improvement or the side effects were pronounced.

In Table II is a summary of the clinical results according to the type of allergic disorder present.

In the sixty-eight patients with perennial allergic rhinitis, the order of symptom disappearance was, in general, as follows: at first nasal pruritus

disappeared; then sneezing stopped; later on, hydrorrhea decreased; and finally nasal obstruction, the most tenacious symptom, abated.

TABLE II. RESULTS OF CLISTIN THERAPY

DISORDER	POOR	FAIR	GOOD	VERY GOOD	EXCELLENT	TOTAL
Perennial allergic rhinitis	2	7	14	35	10	68
Perennial allergic rhinitis with bronchial asthma, etc.	5	4	0	2	1	12
Bronchial asthma		2		1		3
Pruritus or urticaria	2		1	5	1	9
Allergic conjunctivitis			1			1
Seasonal allergic rhinitis					1	1
	9 (10%)	13 (14%)	16 (17%)	43 (45%)	13 (14%)	94

The untoward effects noted in these clinical studies with Clistin consisted of somnolence, lassitude, dizziness, gastric pain, and dryness of the mouth. These side effects were always mild and tolerable, except in two cases where the results were designated as *poor*. One of these patients with a pronounced intolerance to the drug presented a picture of acute intestinal pain which required suspension of treatment on the second day. The other patient experienced an intense stupor, accompanied by dizziness and a feeling of vertigo, following the first dose. In both of these patients, the symptoms disappeared rapidly as soon as the medication was stopped. In only five of the ninety-four patients was the drowsiness produced by Clistin considered to be of a moderate degree and in only one was this side effect classified as severe.

DISCUSSION

Clistin has provided an opportunity to investigate the possibility of selecting chemical configurations which would improve the clinical effectiveness of antihistaminic agents. The pharmacologic activity and clinical effectiveness of Clistin indicate that such a chemical selectivity can lead to an improved antihistaminic agent. Clistin, in addition to having an unusually wide range between the median effective dose and the median lethal dose in animals, has also been found to be effective in low dosage in man and to produce side effects which are almost always mild. The combined pharmacologic and clinical results obtained with Clistin indicate that it is possible to define certain relationships between clinical and pharmacologic activity and chemical configurations of antihistaminic drugs. It would also appear that this approach can lead to improved therapeutic agents as exemplified by Clistin maleate.

CONCLUSIONS

1. Carbinoxamine maleate (Clistin maleate, McNeil) is a new synthetic antihistaminic in which selected chemical configuration has led to a compound which incorporates certain chemical components characteristic of three classes of antihistaminic agents.

2. Pharmacologic studies in animals demonstrate Clistin to have an unusually wide ratio between median effective dose and median lethal dose, indicative of greater antihistaminic potency, as well as of greater safety.

3. Clinical studies have shown Clistin to have a rapid and effective action in the relief of allergic disorders, as well as little toxicity in therapeutic dosage.

4. In its clinical applications, Clistin has proved to be useful in the relief of symptoms caused by perennial allergic rhinopathy and in acute and chronic urticaria and pruritus. In the treatment of bronchial asthma and asthmatic bronchitis, the results were inconsistent and variable.

5. The therapeutic dosage of Clistin varies, in general, between 8 mg. (two tablets) and 16 mg. (four tablets) in a twenty-four-hour period.

6. As the effects are short in duration, it is necessary to administer Clistin every three to four hours.

7. The toxic effects produced by Clistin are almost always mild and, in all cases, transient. They consist of dizziness, somnolence, dryness of the mouth, and gastric pain. Rarely, restlessness and fever have been observed.

8. The possibility of developing more effective antihistaminic agents by chemical selectivity and correlation with pharmacologic and clinical activity is discussed.

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3. Marsh, D. F.: Personal communication.

Addendum.—Since this report was presented, two publications on the Clinical Usefulness of Clistin have appeared in the American literature (Beale, H. D., Rawling, F. F. A., and Figley, K. D.: Clistin Maleate. A Clinical Appraisal of a New Antihistaminic, J. ALLERGY **25**: 521-524, 1954, and Johnson, H. J., Jr.: Clinical Evaluation of a New Antihistaminic: Clistin Maleate, Am. Pract. & Digest Treat. **5**: 862-863, 1954). These clinical studies are in agreement with our results and indicate that Clistin is a potent, effective antihistaminic agent which produces an unusually low incidence of undesirable side effects.

TOXICOLOGIC AND CLINICAL APPRAISAL OF BUCLIZINE, A NEW ANTIHISTAMINIC COMPOUND

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THE search for histamine antagonists of low toxicity and prolonged activity continues. A new class of antihistaminic preparations has been recently synthesized which, in addition to diethylene diamine (piperazine), has three benzylic rings.[1] One of these compounds is buclizine,* 1-p-chlorbenzhydryl-4-p-tertiary butylbenzylpiperazine dihydrochloride; its structural formula is shown in Fig. 1.

In animals, buclizine has been shown to possess marked protective activity against aerosolized and intravenously injected histamine. Although the onset of its action has proved to be relatively slow, the duration of activity is unusually prolonged. For example, it has been shown that 1 mg. per kilogram of the drug administered orally to the guinea pig protected the animal for about sixteen days against the lethal bronchoconstrictor effect of a histamine aerosol.[2] This dose is equivalent to 75 mg. in a man of usual weight, and the persistent action in the guinea pig would lead one to expect very prolonged action in man. Clinical experience does not bear this out, however, as daily doses of 75 mg. or more are well tolerated and appear to have no cumulative effect. This is probably a species difference and deserves further study. In the dog and rat, buclizine is relatively nontoxic,[1] but comparable studies have not been done in the guinea pig.

Preliminary clinical data in normal volunteers and subjects with various allergic states pointed to excellent tolerance for buclizine administered orally. It seemed desirable to determine whether or not these advantageous properties would be evident after long-term administration of the drug and to gather further data on its therapeutic efficacy.

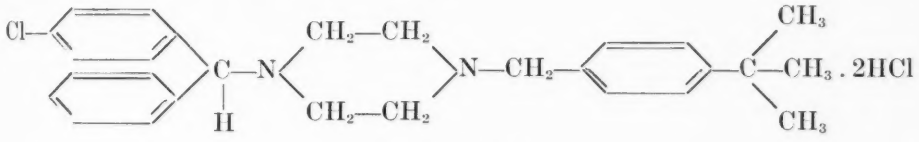

Fig. 1.—Buclizine.

MATERIALS AND METHODS

Seventy patients were given oral buclizine in the period extending from Dec. 7, 1953, to Dec. 18, 1954. Among the seventy patients, twenty-eight were

From the Allergy Service, Newton-Wellesley Hospital, and the Division of Clinical Immunology, the Evans Memorial Hospital, the Massachusetts Memorial Hospitals, and the Department of Medicine, Boston University School of Medicine.

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*Kindly supplied by Chas. Pfizer & Company, Inc., Brooklyn, New York.

male and forty-two female; their ages ranged from 9 to 73 years, with an average age of 35 years. Of these patients, forty-two had vasomotor rhinitis (as far as can be determined, there was no evidence of an allergic cause), twenty-two had allergic rhinitis of the perennial variety, and six had urticaria. These conditions lend themselves well to a study of this type, for such patients frequently require daily medication for long periods in an effort to obtain relief. All patients had moderate to severe symptoms and had often failed to obtain relief by other measures, including injections of allergens. The patients who were receiving injections (22 per cent) continued to do so, but were urged to avoid other antihistaminic drugs. Seventy-five milligrams of buclizine per day was prescribed, to be taken in three equally divided doses between arising in the morning and bedtime. Patients were encouraged to take the medication irrespective of "need" unless untoward reactions occurred. In some, the daily dose had to be reduced to 25 or 50 mg. per day, and in children the daily dosage was one-half the adult dose. There was no way of being absolutely certain about the number of days the subjects were on the drug but, based on their statements, the compound was taken orally for from 8 to 233 days, with an average of 93 days. Since the primary purpose of the study was to determine the presence or absence of toxic effects from prolonged administration of the drug, neither a placebo control nor a blind study was attempted.

Prior to and following the administration of buclizine, urinalyses, van den Bergh reactions, thymol turbidity tests, studies of the hemoglobin levels, and leukocyte and differential counts were obtained in twenty patients; fifteen patients had urinalyses and blood studies; and in fifteen it was possible to record only the urinary findings. These fifty patients comprised the experimental group. The laboratory procedures were not satisfactorily completed in the remaining twenty patients and, therefore, are not included in this group. The method for the van den Bergh reaction was a modification of that described by Evelyn and Malloy.[3] Bilirubin is converted to a red-violet azobilirubin when treated with Ehrlich's reagent (diazotized sulfanilic acid). A fifteen-minute reading is obtained in aqueous solution, giving the total direct-reacting bilirubin, and a thirty-minute reading is taken in methyl alcohol solution. This reading constitutes the total bilirubin. The difference between the two readings is a measure of the indirect-reacting bilirubin. The method for thymol turbidity was a modification of that described by Maclagan.[3] Under certain conditions, a turbidity is formed when serum is treated with a buffered thymol solution due to the precipitation of a protein-thymol-phospholipid complex. The density of the turbidity is determined nephelometrically.

Laboratory examinations were repeated at approximately four- to eight-week intervals and in several instances this period had to be extended. All subjects were seen at seven- to fourteen-day intervals over a period of weeks to months, and were observed carefully for the development of possible toxic reactions. The effectiveness of the buclizine in relieving symptoms was noted.

RESULTS OF LABORATORY STUDIES

The fifty patients comprising the experimental group were given oral doses of buclizine daily for an average of approximately four months. One patient

took buclizine for 233 days, and none received the drug for less than ten weeks. Of the fifty patients in whom the urinalyses were followed at stated intervals, evidence of renal dysfunction was found in none. This was based both on the absence of significant glycosuria or albuminura and on examination of centrifuged sediments, which did not reveal abnormal microscopical elements. Although concentration tests were not included, all patients were able, at one time or another, to concentrate urine to a specific gravity of 1.020 or more.

Evaluation of hepatic function in twenty patients was based on the van den Bergh reaction and the thymol turbidity test. These examinations were carried out on fasting blood samples. No abnormalities in function, as determined by these two tests, were observed before or during the course of therapy with buclizine.

Of the thirty-three patients in whom hemoglobin determinations and complete blood counts were made periodically, evidence of anemia, leukopenia, or abnormalities of the microscopic picture of the blood was not observed.

SIDE EFFECTS

Of the seventy patients given buclizine, fifty-two (74 per cent) were free from side effects. The reactions in the remaining eighteen (26 per cent) are summarized in Table I.

TABLE I. SIDE EFFECTS PRODUCED IN SEVENTY PATIENTS BY BUCLIZINE

REACTION	NO. OF PATIENTS AFFECTED
Nausea	1
Dizziness	1
Drowsiness	6
Headache	1
Dryness of the mouth	8
Urticaria "aggravated"	1
Total side effects	18 (26 per cent)

Of the six patients experiencing drowsiness, in none was it especially troublesome, and complete or almost complete disappearance of the sedative effect followed a decrease in the dose of the drug. All patients except one were able, within a few weeks, to tolerate full doses of the drug. Dryness of the mouth in eight patients was relieved as the dose of the drug was reduced by approximately 50 per cent. In the remaining four patients (6 per cent), the drug was discontinued. These had dizziness, headache, and nausea, and one patient stated that the urticaria for which she was taking the drug became worse. This statement was difficult to assess. Palpitation, insomnia, jitteriness, nervousness, headache, and digestive disturbances were not noted.

THERAPEUTIC RESPONSE

The results were evaluated according to the following criteria: *excellent* if complete relief of symptoms occurred and lasted for several hours; *moderate* if relief was greater than 50 per cent and lasted for two or more hours; *slight* when there was 25 to 50 per cent relief; and *no relief* when relief was less than

25 per cent or the duration of relief was thirty minutes or less. The degree of relief is shown in Table II. Relief of symptoms, when it occurred, required about forty-five to sixty minutes.

TABLE II. THERAPEUTIC RESPONSE TO BUCLIZINE

DIAGNOSIS	NONE	SLIGHT	MODERATE	EXCELLENT	TOTAL NUMBER OF CASES
Perennial Allergic Rhinitis	3	4	7	8	22
Vasomotor Rhinitis	8	7	13	14	42
Urticaria		1	3	2	6

Of twenty-two patients with perennial allergic rhinitis, fifteen (68 per cent) reported moderate to excellent relief of symptoms; in four, relief was slight; and no relief was seen in the remaining three. Relief was moderate to excellent in twenty-seven (64 per cent) of the forty-two patients with vasomotor rhinitis; seven had only slight relief; and no relief was obtained in eight. Five of the six patients with urticaria experienced moderate to excellent relief and in one the symptoms, as stated previously, apparently were intensified. Increasing the dose of buclizine in the patients who failed to obtain more than slight improvement seemed to make little difference in the effectiveness of the drug.

Forty-seven subjects had moderate to excellent relief and in thirty-two this persisted for two to four hours; in eight it was five to eight hours; and in the remaining seven it was ten or more hours.

DISCUSSION

Clinical experience, including simple studies of the renal, hepatic, and hematologic systems, appears to support animal experimental data showing that the drug can be administered for weeks or months without producing toxic manifestations. In this rather small group of patients, granulocytopenia and anemia were not observed, effects, which, on occasions, have been reported following the use of other histamine-antagonizing agents.[4-8] An additional feature, aside from toxicity, was the absence of serious side effects. Dryness of the mouth and drowsiness were easily controlled by decreasing the dose of the drug without significant loss of clinical effectiveness. In the majority of instances, these mildly annoying side effects gradually subsided as treatment was continued, to be followed frequently by complete tolerance of full doses of the drug. Excellent tolerance by patients for buclizine was confirmed by another group of investigators,[9] who found a lessened incidence of side reactions when the drug was compared with most other antihistaminic preparations.

Despite the somewhat slower onset of action, buclizine appears, in the majority of cases, to control very satisfactorily the symptoms of perennial allergic rhinitis, vasomotor rhinitis, and urticaria.

The duration of action of the drug deserves comment in view of the discrepancy between experiments in animals and clinical experience. Protection from aerosolized histamine was observed for as long as sixteen days following a single dose of 1 mg. per kilogram in guinea pigs[2] and in similar experiments in

another report this protection was observed for four days following a single dose of 2.5 mg. per kilogram.[1] Even this represents prolonged action when the metabolic rate of an animal as small as the guinea pig is taken into account. In this animal, therefore, this prolonged action would lead one to expect a cumulative effect of the drug and the development of toxic manifestations with daily doses of the order given to patients. However, such doses in rats, dogs,[1] and in man (75 to 100 mg. daily) do not produce toxic reactions and, furthermore, in most patients clinical effectiveness does not persist for more than a few hours.[9] These observations suggest that the excretion or metabolism of the drug in guinea pigs is different from that of rats, dogs, and man. If this is indeed correct, then we have an example of the part played by species difference in the behavior of drugs.

SUMMARY

1. Buclizine, 1-p-chlorbenzhydryl-4-p-tertiary butylbenzylpiperazine dihydrochloride, a new antihistaminic drug, was administered in seventy patients with allergic and related states, with the majority of these patients under observation for many months.

2. Buclizine, taken orally in doses of 25 to 75 mg. daily for an average of four months, produced no significant abnormalities in the formed blood elements or in hepatic and renal function.

3. In contrast to the prolonged activity of the drug in guinea pigs, the duration of action of comparable dosage in man in relatively short—a point deserving further study.

4. The drug appeared to be effective is relieving the symptoms in the majority of patients with perennial allergic rhinitis, vasomotor rhinitis, and urticaria.

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A COMPARATIVE STUDY OF Sc-4959 (1-n.-propyl-3-ethyl-5-chloro-6-aminotetrahydropyrimidinedione) AND HYADRINE IN BRONCHIAL ASTHMA

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EPHEDRINE, theophylline, and their analogues remain the mainstay of drug therapy in bronchial asthma notwithstanding the advent of corticotropin (ACTH) and the adrenal cortical steroids. These well-established agents unfortunately cause unpleasant effects, referable to the central nervous, cardiovascular, and gastrointestinal systems, which interfere with their use in a considerable percentage of patients. Though it has been possible to curtail some of the undesirable reactions by combining these drugs with sedatives, antihistamines, and medication designed to reduce gastric disturbances, attempts to produce potent, yet better-tolerated agents for use in bronchial asthma continue.

Two new agents have been made available for clinical trial. One of these is a uracil derivative (1-n.-propyl-3-ethyl-5-chloro-6-aminotetrahydropyrimidinedione), hereafter referred to as Sc-4959. For the structural formula see Fig. 1. In the experimental animal the drug has many of the activities of theophylline, but less emetic action.[1]

When Sc-4959 was given intravenously in doses up to 150 mg. to patients with emphysema, mitral stenosis, and hypertension the observed effects were similar to those occurring following aminophylline, except that disagreeable side effects were not seen.[1]

Preliminary clinical data in subjects with bronchial asthma following single oral doses of 50 mg. of the drug have indicated significant bronchodilator activity with an increase in vital and maximum breathing capacities.

The second agent is Hyadrine, an oral tablet containing diphenhydramine 37.5 mg., aminophylline 150.0 mg., and racephedrine hydrochloride 25.0 mg. Considerable experience[2] with a closely similar preparation,* differing from Hyadrine only in that the dose of diphenhydramine is 25.0 mg. and that of aminophylline is 100.0 mg., has shown this combination of drugs to be well tolerated and useful for the relief of bronchial asthma. The amount of diphenhydramine and aminophylline was increased in Hyadrine with the hope that greater effectiveness could be achieved and that the occurrence and severity of side effects incident to diphenhydramine would not be significantly in-

From the Evans Memorial, Massachusetts Memorial Hospitals, the Department of Medicine, Boston University School of Medicine, and the Allergy Service of the Newton-Wellesley Hospital.

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*Diphenhydramine, 25.0 mg.; aminophylline 100.0 mg.; and racephedrine 25.0 mg.; supplied as Hydryllin with Racephedrine, G. D. Searle & Co., Chicago, Ill.

creased. Furthermore, the inclusion in this study of Hyadrine, the action of which could in some degree be predicted, was helpful in assessing the aforementioned drug, Sc-4959.

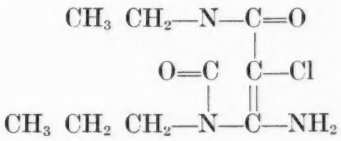

Fig. 1.—Chemical structure of Sc-4959.

MATERIALS AND METHODS

Between Sept. 3, 1954, and May 20, 1955, 90 patients with bronchial asthma served as subjects for this study. There were 57 men and 33 women, ranging in age from 9 to 72 years, with an average age of 36 years. Seventy-six patients had the perennial type of asthma; 6 had both seasonal and perennial asthma, and 8 had seasonal asthma. Based on the frequency, severity, duration, the daily need of medication, as well as on a number of pulmonary function tests, all patients were having mild to moderately severe asthma at the time treatment with one or another of the preparations was started. Two patients were taking either cortisone or hydrocortisone during the period of the investigation. Some patients (52 per cent) were receiving treatment with specific allergenic extracts and many had been instructed to eliminate offending allergens. The patients receiving injections continued to do so but, like all the patients in the study, were urged to avoid the use, unless otherwise instructed, of other medications designed for the relief of asthma.

The drugs* included were the following: (1) Hyadrine (diphenhydramine 37.5 mg.; aminophylline 150 mg.; and racephedrine hydrochloride 25 mg.); (2) Sc-4959, 100 mg.; (3) placebo (lactose). They were packaged in similar appearing bottles (50 tablets per bottle) and were identified by a code unknown to the physician. Though each of the 3 preparations was identical in appearance and taste, it was determined at the conclusion of the study that there was a distinct odor of menthol associated with the placebo and Sc-4959. Comments concerning this were so few as to indicate that this played little part in the evaluation of the drugs.

In order to enlist the cooperation of the subjects, it was necessary to identify the tablets as medication intended for the relief of asthma. It was felt that the expectation of relief would bias the results equally for the placebo and the drugs. To ensure that the subjects be given a trial with each of the 3 preparations, the drugs were arbitrarily dispensed in rotation, but the order was varied as shown in Table I.

The prescribed dose was the same for all preparations; namely 1 tablet every 4 hours as needed. One or more weeks were allowed for evaluation of each preparation by each patient. For one or another reason, it was not possible, in all instances, to adhere to this schedule. As might be expected, a number of patients became aware of the comparative nature of the study.

*Kindly supplied by the G. D. Searle & Co., Chicago, Ill.

TABLE I. ORDER OF ADMINISTRATION OF THE MEDICATION

GROUP 1	GROUP 2	GROUP 3
Hyadrine	Sc-4959	Placebo
Sc-4959	Placebo	Hyadrine
Placebo	Hyadrine	Sc-4959

All subjects were seen at 7- to 21-day intervals over a period of weeks to months, and, based on their statements, the results were classified as follows: marked, if complete or almost complete relief of symptoms occurred for 4 or more hours; moderate, if relief was 50 to 75 per cent and lasted 2 or more hours; slight, if relief was between 25 and 50 per cent and lasted 1 to 2 hours; and none, if relief was less than 25 per cent or if the duration of activity was short. The patients were questioned carefully for possible untoward reactions. The grading of the patients' responses was based on two subjective judgments, (1) the patient's own opinion and (2) the physician's (personal) interpretation of the patient's statement. The subjective element of both should, nevertheless, apply equally to the three medications.

A total of 64 patients used all 3 preparations and comprised the experimental group. The remaining 26 patients had failed to complete a trial with all 3 medications and, therefore, were not included when the data were subjected to statistical analysis. However, the experience of all 90 patients was utilized when the side effects were considered.

RESULTS

Table II shows the frequency of response for each degree of relief by the 64 patients in the experimental group. The subscript 1 indicates the drug given first in the sequence.

TABLE II. FREQUENCY DISTRIBUTION BY DRUG AND ORDER GIVEN

DRUG		DEGREE OF RELIEF				TOTAL
		MARKED	MODERATE	SLIGHT	NONE	
Hyadrine Response	H_1	18	4	0	1	23
	S_1	14	6	0	3	23
	P_1	9	7	1	1	18
Total for all patients		41	17	1	5	64
Sc-4959 Response	H_1	7	4	5	7	23
	S_1	3	5	2	13	23
	P_1	2	2	7	7	18
Total for all patients		12	11	14	27	64
Placebo Response	H_1	3	2	3	15	23
	S_1	1	3	1	18	23
	P_1	1	1	3	13	18
Total for all patients		5	6	7	46	64

Mere inspection of the data clearly shows that it is only among those patients receiving Hyadrine that a large proportion derived marked relief; and, conversely, a high proportion of those given the placebo derived no benefit. Sc-4959 occupies an intermediate place with respect to effectiveness.

STATISTICAL ANALYSIS*

To establish, formally, the presence or absence of statistical differences, the response scale was arbitrarily assigned the values 3 (marked relief), 2 (moderate relief), 1 (slight relief), 0 (no relief) and a mean "index of perceived effectiveness" computed for each drug for each group of patients ("group" is defined by the order in which the drug was given). Mean values are shown in Table III.

TABLE III. INDICES OF PERCEIVED EFFECTIVENESS BY DRUG AND ORDER GIVEN

	H_1	S_1	P_1	ALL PATIENTS
Hyadrine	2.70	2.35	2.33	2.47
Sc-4959	1.48	.91	.94	1.13
Placebo	.70	.43	.44	.53
All drugs	1.62	1.23	1.24	1.375

For graphic presentation, the above means are plotted in Fig. 2. It is obvious from the graph that the effectiveness of the drugs is perceived at three different levels by the patients, and the position of the drug in the order of rotation was without influence.

The analysis of variance in Table IV confirms the evidence previously noted; the differences among drugs are highly significant, while all other effects are nonsignificant.

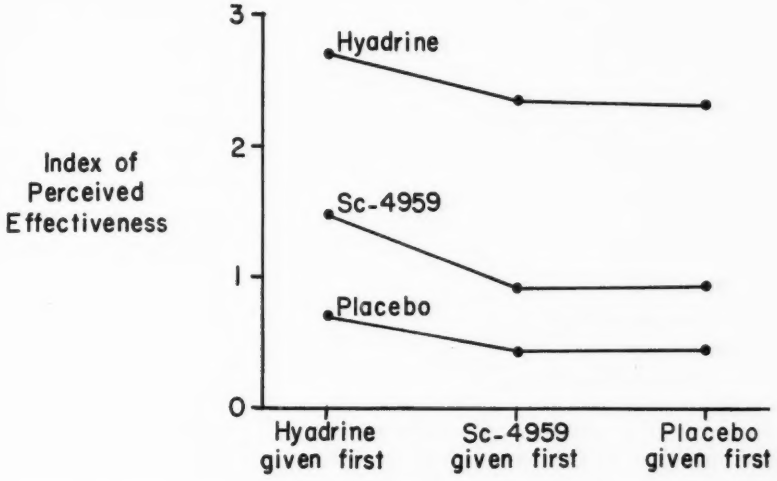

Fig. 2.—Graph showing mean indices of perceived effectiveness by drug and order of administration.

To evaluate the difference between Sc-4959 and the placebo, a *t*-test is appropriate. Using the estimate of residual error obtained in Table IV, $t = 3.60$ is obtained, a highly significant value. As a check, and to gain further insight into the perceived differences between the placebo and Sc-4959, a table of differences for all patients was obtained (Table V).

*The statistical analysis was carried out by Mr. John E. Alman of the Office of Statistical and Research Services, Boston University.

TABLE IV. ANALYSIS OF VARIANCE OF INDICES OF EFFECTIVENESS

SOURCE	SUM OF SQUARES	D.F.	MEAN SQUARE	F
Total	317.00	191		
Drugs	126.13	2	63.07	81.9 ($P<.001$)
Order	6.64	2	3.32	
Drug x Order	.67	4	.17	
Subjects	89.69	61	1.47	
Residual	93.87	122	.77	

Fisher's t-test, which tests the hypothesis that the true mean difference is zero, when applied to the above differences yields $t = 4.01$. This confirms the previous t-test as to the significance of the average difference between Sc-4959 and placebo. Though there is a real difference between Sc-4959 and placebo, its perceived effectiveness, on the average, must be recorded as "slight." It should be noted that less than one-half of the patients gave an advantage to Sc-4959, and that its over-all average index, which is 1.13, is just over the "slight improvement" scale point.

TABLE V. DIFFERENCES BETWEEN SC-4959 AND PLACEBO RATINGS BY 64 PATIENTS

Difference	+3	+2	+1	0	−1	−2	−3
Frequency	7	5	17	29	4	1	1

The frequency distribution of perceived difference between Hyadrine and Sc-4959 is shown below in Table VI. In contrast to Sc-4959, Hyadrine has an index midway between "moderate" and "marked" improvement, indicating a substantially higher degree of perceived effectiveness.

TABLE VI. DIFFERENCE BETWEEN HYADRINE AND SC-4959 RATINGS BY 64 PATIENTS

Difference	+3	+2	+1	0	−1	−2	−3
Frequency	17	11	18	12	6	0	0

Forty-six out of 64 patients (72 per cent) indicated Hyadrine to be more effective than Sc-4959, and 28 out of the 64 patients (44 per cent) indicated it to have a marked advantage (+2 or +3). The t-test applied to the above data yields $t = 8.1$, a highly significant value.

SIDE EFFECTS

Of the 80 patients given Hyadrine, 51 (64 per cent) had no side effects. The reactions in the remaining 29 (36 per cent) are summarized in Table VII. Sixteen patients described sedation (drowsiness and fatigue); 11 reported central nervous system stimulating effects (nervousness, insomnia, palpitation, and headache), and 6 had untoward reactions involving the gastrointestinal tract (nausea, "bad taste" and dryness of the mouth). In only 5 patients were these effects serious enough to warrant discontinuance of the drug, indicated as "marked" in Table VII.

Among the 70 patients given Sc-4959, 53 (76 per cent) reported no side effects. The reactions in the remaining 17 patients (24 per cent) are listed in Table VIII. Of the patients with side effects, 13 had symptoms referable to

TABLE VII. SIDE EFFECTS PRODUCED IN 80 PATIENTS BY HYADRINE*

TYPE	SLIGHT	MODERATE	MARKED
Nervousness	5	1	1
Bad taste	1		
Insomnia	1	1	
Drowsiness	8	4	3
Palpitation			1
Nausea	3	1	
Headache		1	
Fatigue	1		
Dryness of mouth		1	

*See text.

stimulation of the central nervous system (palpitation, headache, and dizziness), 7 had nausea, and 1 each reported urticaria and drowsiness. One patient stated that his asthma, for which he was taking the drug, became worse, and, therefore, it was omitted. All untoward reactions were either slight or moderate.

TABLE VIII. SIDE EFFECTS PRODUCED IN 70 PATIENTS BY Sc-4959*

TYPE	SLIGHT	MODERATE	MARKED
Headache	3	2	
Dizziness	3	2	
Nausea	4	3	
Urticaria		1	
Palpitation	3		
Aggravation of asthma		1	
Drowsiness	1		

*See text.

Of the 68 who received the placebo, 63 (93 per cent) were free of side reactions. The reactions in the remaining 5 patients were moderate, and are shown in Table IX. The medication was stopped in one patient who, like the one mentioned above receiving Sc-4959, stated that his asthma was aggravated. Two patients developed nausea, and 1 each reported drowsiness and fatigue. This frequency of placebo reactors was less than anticipated.

TABLE IX. SIDE EFFECTS PRODUCED IN 68 PATIENTS BY PLACEBO*

TYPE	SLIGHT	MODERATE	MARKED
Drowsiness		1	
Nausea		2	
Fatigue		1	
Aggravation of asthma		1	

*See text.

DISCUSSION

Since, in the response scale, 46 out of the 64 patients (72 per cent) gave the placebo a zero rating, and 41 out of the 64 patients (64 per cent) gave Hyadrine a top rating, there seems to be no question but that Hyadrine was an effective agent in relieving the symptoms of bronchial asthma. Only 6 patients (9 per cent) given Hyadrine reported it as producing slight or no improvement. Sc-4959 occupied an intermediate position with respect to effectiveness, despite the fact that a majority of the patients (64 per cent) reported it as pro-

ducing slight or no improvement. However, with Sc-4959, approximately twice as many patients obtained marked or moderate improvement as did with the placebo. Though the statistical analysis shows Sc-4959 to have a significant advantage over the placebo in the treatment of bronchial asthma, it certainly is not perceived to be nearly as effective as Hyadrine. The number of patients, 11 (17 per cent), claiming marked to moderate relief with the placebo was considerably less than that expected on the basis of other studies.[3, 4]

The order of administration of the drugs was not a significant factor. Hyadrine first produced somewhat higher indices (see Table III), which might lead to the surmise that the use of an effective drug first produced a "halo" effect that carried over to the others. There is no statistical evidence to back up this surmise, however, and it can be explained equally well by random sampling variation.

As a sidelight of interest, if the assumption is made that those 11 patients claiming relief from the placebo are unreliable and therefore eliminated, the indices of perceived effectiveness obtained are as follows: Hyadrine, 2.47; Sc-4959, 0.77; placebo, 0.13. The index for Hyadrine is unchanged, but the indices for Sc-4959 and placebo are reduced considerably from those given in Table III. This is, intuitively at least, added evidence that Sc-4959 can only be labeled as "slightly" effective, if at all, as an agent for the relief of asthma. However, such "handling" of the data is contrary to good statistical practice, hence this conclusion can only be treated as a speculation.

SUMMARY

Sixty-four patients compared the effectiveness of three preparations in bronchial asthma. These were Sc-4959 (1-n.-propyl-3-ethyl-5-chloro-6-aminotetrahydropyrimidinedione 100 mg.), Hyadrine (diphenhydramine 37.5 mg., aminophylline 150.0 mg., and racephedrine 25.0 mg.), and a placebo (lactose). The results were subjected to statistical analysis. Hyadrine was found to be a very effective agent in the management of bronchial asthma, with a clear-cut advantage over Sc-4959 and the placebo. Though Sc-4959 exhibited some advantage over the placebo, the results in bronchial asthma were disappointing. The number of patients who scored the placebo as affording marked to moderate relief were considerably less than that expected. The order of administration of the drugs had no appreciable effect on the results. The most effective preparation, Hyadrine, also gave the highest incidence of side effects, but these, in most instances, were mild and unimportant.

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3. Lowell, F. C., Schiller, I. W., Alman, J. E., and Mountain, C. F.: The Antihistaminic Drugs in the Treatment of the Common Cold, New England J. Med. **244:** 132, 1951.
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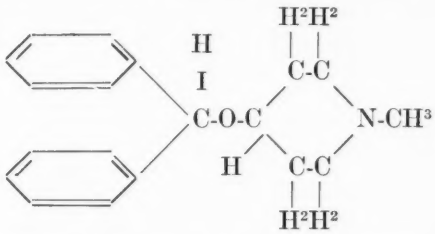
CLINICAL EVALUATION OF DIPHENYLPYRALINE*

HENRY B. NACHTIGALL, M.D., NEW YORK, N. Y.

THIS clinical investigation of Diphenylpyraline, a new antihistaminic drug, reveals a high degree of effectiveness, with a practically negligible incidence of side effects, which warrants introduction of the drug for clinical use. Salter,[1] in referring to the work of Loveless and Dworin,[2] says that probably all the antihistamine drugs used by them would be equally effective, were it not for the untoward effects. The superiority of one antihistamine over the others, therefore, is dependent not solely upon its potency, but upon its effectiveness without intolerable side reactions.

The chemical formula for Diphenylpyraline is 1-methyl-piperidyl 1-4 benzhydryl ether, and it has the following structural formula:



A previous clinical evaluation of Diphenylpyraline[3] concerned its effectiveness in ragweed pollinosis. This report is on the use of Diphenylpyraline for the relief of allergic symptoms in 121 patients from the Allergy Clinic of the New York Eye and Ear Infirmary. Patients were of both sexes, ranging in age from 5 to 64 years. Of these, thirty-six had seasonal allergic rhinitis, fifty-eight had perennial allergic rhinitis, eighteen had allergic conjunctivitis, and nine had bronchial asthma, representing a typical cross section of those allergic conditions seen in everyday practice.

The patients with seasonal allergies were those with proved sensitivity to plantain and timothy grasses. Those with perennial allergic rhinitis had sensitivity of the house dust type. All these patients had undergone, or were undergoing, active hyposensitization when studied, and were selected for treatment from a much larger group because of the persistence of allergic symptoms. Thus, Diphenylpyraline was used, as all antihistaminic agents should be used in these conditions, as adjuvant therapy.[5]

Patients were seen once weekly and were given two identical, but coded, envelopes. One contained 2 mg. tablets of Diphenylpyraline, and the other contained identically appearing placebo tablets. Patients were initially told to

Received for publication April 13, 1955.
*Made available by Schenley Laboratories, Inc.

take one tablet every four hours if necessary. Subsequently, dosage was adjusted to the individual needs of the patient, or the drug was discontinued in those who obtained no relief.

Each envelope contained a sufficient number of tablets for approximately one-half week (three and one-half days). The patient was instructed to finish the tablets in one envelope before starting the second, and to note daily the degree of relief and side effects on a diary form supplied to the patient. These forms were evaluated by the investigator during the weekly visits.

RESULTS

The data are summarized in Table I. Of a total of 121 patients who received the drug, eighty-six, or 71 per cent, obtained excellent to moderate relief of symptoms. The results were extremely satisfactory in both seasonal and perennial allergic rhinitis, with excellent to moderate relief of symptoms in 88.8

TABLE I

ALLERGIC CONDITION		NUMBER OF CASES	RELIEF OF SYMPTOMS: EXCELLENT TO MODERATE	RELIEF OF SYMPTOMS: SLIGHT TO NONE	PER CENT RELIEVED	UNTOWARD REACTIONS	PER CENT UNTOWARD REACTIONS
Allergic rhinitis (seasonal)	Drug	36	32	4	88.8	2*	5.5
	Placebo	23	2	21	8.7	1†	4.4
Allergic rhinitis (perennial)	Drug	58	44	14	75.8	1‡	1.7
	Placebo	21	4	17	19.0	0	0
Allergic conjunctivitis	Drug	18	6	12	33.3	1*	5.5
	Placebo	5	1	4	20.0	0	0
Bronchial asthma	Drug	9	4	5	44.4	0	0
	Placebo	6	1	5	16.6	0	0
Drug-treated		121	86	35	71.0	4	3.3
Placebo-tested		55	8	47	14.5	1	1.8

*Slight drowsiness.
†This patient had slight drowsiness with both placebo therapy and Diphenylpyraline.
‡Slight dizziness.

per cent and 75.8 per cent of patients, respectively. The percentages of patients with allergic conjunctivitis and bronchial asthma relieved by Diphenylpyraline were comparable to the results obtained by other investigators[1, 4] in their studies.

Because of the variation of allergic symptoms that may occur from day to day, close attention was given to the differentiation between relief due to the drug, and relief as a result of this variation. Placebo studies may aid in this differentiation, but only partially. In fifty-five patients who received placebo therapy, eight, or 14.5 per cent, had excellent to moderate relief of symptoms. It should be noted also that observations of both drug and placebo effect were necessarily of short duration in those patients with limited seasonal allergic diseases.

Diphenylpyraline showed a remarkable paucity of side effects. Only four patients, or 3.3 per cent of the total, had any complaints that could be attributed to the drug. Two patients with seasonal allergic rhinitis and one with allergic

conjunctivitis complained of slight drowsiness. It should be noted that one of the two with seasonal allergic rhinitis had the same complaint on placebo therapy. One patient with perennial allergic rhinitis complained of slight dizziness.

The minimum trial period for each patient was two weeks, but the majority of patients received the drug for at least eight weeks. No complaints referable to possible toxicity were elicited from any patients, and examinations during the weekly visits also did not elicit any evidence of toxicity.

DISCUSSION

The optimum dosage for the majority of patients was 2 mg. three times a day. The extreme effective dosage range of the drug, from our investigation, appeared to be 4 to 8 mg. daily in divided doses. It is to be noted, however, that since adjuvant therapy implies a selective decision by both patient and physician, it is conceivable that with allergic symptoms of a slight degree 2 mg. per day would also be effective.

Perusal of the literature reveals that not only is Diphenylpyraline over-all as effective an antihistaminic as any of the reported antihistaminic preparations, but that, in addition, it has a remarkable paucity of side effects, none of which ever necessitated discontinuance of the drug.

CONCLUSIONS

1. Diphenylpyraline, a newer antihistaminic compound, was evaluated clinically in 121 patients with allergic disorders.

2. Eighty-six, or 71 per cent, of the patients experienced excellent to moderate relief of allergic symptoms with Diphenylpyraline.

3. The extreme effective dosage range was 4 to 8 mg. or Diphenylpyraline in divided doses daily. The optimum dosage was 2 mg. three times a day.

4. Over-all side effects occurred in only four, or 3.3 per cent, of the patients tested, one of whom had the same side effects with placebo therapy.

5. Diphenylpyraline is a potent and effective antihistiminic agent with an extremely small incidence of negligible side effects, and should enjoy extensive clinical use as a valuable addition for the treatment of allergic diseases.

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1. Salter, William T.: Textbook of Pharmacology, Philadelphia, 1952, W. B. Saunders Company.
2. Loveless, M. H., and Dworin, M.: Allergy and Antihistamine Therapy, Bull. New York Acad. Med. **25**: 473-487, 1949.
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4. Spain, W. C., and Pflum, F. A.: Evaluation of Present Status of Antihistaminic Substances, New York J. Med. **48**: 2272-2275, 1948.
5. Sherman, W. B.: The Uses and Abuses of Antihistaminic Drugs, Bull. New York Acad. Med. **27**: 309-324, 1951.

American Academy of Allergy

PRECONVENTION SCIENTIFIC ASSEMBLY

Saturday and Sunday
Feb. 4 and 5, 1956
The Chase Hotel
St. Louis, Missouri

UNDER THE AUSPICES OF THE AMERICAN ACADEMY OF ALLERGY AND SPONSORED IN PART THROUGH A GRANT BY CIBA PHARMACEUTICAL PRODUCTS, INC.

There will be three panel discussions reviewing the status of therapeusis in allergic diseases. The panelists are invited guests, fellows, and members of the American Academy of Allergy, each especially and eminently qualified in a particular field of medicine.

There will be no charge or registration fee for attending these meetings. To aid in planning future Post-Graduate Reviews, those in attendance will be asked to register.

Indications and Contraindications for Therapy in Bronchial Asthma

9:00 A.M. Saturday, Feb. 4, 1956
The Regency Room

Moderator: Leo H. Criep, M.D., Associate Professor of Medicine and Chief of the Allergy Clinic, School of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania.

Participants:

Harry L. Alexander, M.D., Professor Emeritus, Clinical Medicine, Medical School, Washington University, St. Louis, Missouri.

Alvin L. Barach, M.D., Clinical Professor of Medicine, College of Physicians and Surgeons, Columbia University; Associate Attending, Presbyterian Hospital, New York, New York.

Samuel C. Bukantz, M.D., Associate Professor of Clinical Medicine, Medical School, Washington University; Consultant in Allergy, Ward Service, Barnes Hospital; Consultant in Allergy, Veterans Administration Hospital, St. Louis, Missouri.

T. S. Danowski, M.D., Renziehausen Professor of Research Medicine, School of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania. (By invitation.)

John C. Krantz, Jr., Ph.D., D.Sc., Professor of Pharmacology, School of Medicine, University of Maryland, Baltimore, Maryland. (By invitation.)

Louis Weinstein, M.D., Associate Professor of Medicine, Boston University School of Medicine, Boston, Massachusetts. (By invitation.)

The discussion will be informal, supplemented by the distribution of outlines to aid audience participation.

DRUGS: Mechanism of action and of relief—sympathomimetic; iodides; aminophyllin; antihistamines.

ANTIBIOTICS: Indications, contraindications, untoward results.

STEROIDS: Displacement, replacement, pharmacologic effects; indications, contraindications; Dosage; use in status asthmaticus.

ADJUVANT THERAPY: Intermittent positive pressure breathing; intravenous fluids; oxygen; mucolytic agents; aerosols.

MISCELLANEOUS: Role of acetylcholine management of the asthmatic infant and child, cardiac asthma, bronchostenosis, pulmonary tumor; foreign body; pulmonary emphysema; pulmonary fibrosis.

Immunological and Clinical Aspects of Antigen Therapy

2:00 P.M. Saturday, Feb. 4, 1956
The Regency Room

Moderator: Oscar Swineford, Jr., M.D., Professor of Internal Medicine (Allergy-Arthritis Division), University of Virginia Medical School, Charlottesville, Virginia.

Participants:

J. Harvey Black, M.D., Dallas, Texas.

Samuel M. Feinberg, M.D., Professor of Medicine and Director of Allergy Research Laboratory, Northwestern University Medical School, Chicago, Illinois.

William B. Sherman, M.D., Associate Professor of Medicine, Columbia University; Attending Physician, Roosevelt Hospital; Associate Attending Physician, Presbyterian Hospital, New York, New York.

INTRODUCTION.—Dr. Swineford.

CRITICAL REVIEW OF DESENSITIZATION.

Experimental Background: Dr. Swineford.
Clinical Background: Dr. Sherman.

Intermission

PRACTICAL CLINICAL CONSIDERATIONS.

Indications for Antigen Therapy: Dr. Black.

Selections of Antigens: Dr. Black. (Criteria, mixtures, and separate antigens, stock vs. autogenous, food, inhalant, bacterial.)

Preparation of Antigens: Dr. Black. (General principles, antigen sources, usual methods, variations, standardization.)

Methods of Treatment: Dr. Feinberg. (General schemes—pre-seasonal, seasonal, perennial; initial and maximal doses; increments, frequency of injections; routes—intradermal, subcutaneous, oral, aerosol; duration of treatment, criteria for stopping.)

Reactions: Dr. Feinberg. (Significance, prevention, treatment.)

Intermission

QUESTIONS AND ANSWERS

(Those who plan to attend are urged to mail questions to Oscar Swineford, Jr., M.D., University of Virginia Hospital, Charlottesville, Virginia.)

The Association of Allergists for Mycological Investigations

9:00 A.M. Sunday, Feb. 5, 1956
Starlight Roof

Chairman: **Homer E. Prince, M.D.**
Houston, Texas.

Assistant Chairman: **Sim Hulsey, M.D.**
Fort Worth, Texas.

Program*

PATCH TESTING WITH THE ETHER-SOLUBLE FRACTION OF MOLD PELLICLES. Stephan Epstein, M.D., Marshfield Clinic, Marshfield, Wisconsin, and Clinical Associate Professor of Dermatology, University of Minnesota Medical School; Homer E. Prince, M.D., Clinical Professor of Medicine, Baylor University College of Medicine, Houston, Texas; Marie B. Morrow, Ph.D., Associate Professor of Botany and Mycologist, Plant Research Institute, The University of Texas, Austin, Texas.

CLASSIFICATION OF AIR-BORNE BACTERIA: ISOLATION AND IDENTIFICATION. Marie B. Morrow, Ph.D., Associate Professor of Botany and Mycologist, Plant Research Institute; George H. Meyer, M.A.; and James E. Grimes, B.A., The University of Texas, Austin, Texas.

CLINICAL SIGNIFICANCE OF AIR-BORNE BACTERIAL ALLERGENS. L. O. Dutton, M.D., El Paso, Texas.

STUDY OF THE ALLERGENICITY BY CROSS NEUTRALIZATION EXPERIMENTS WITH EXTRACTS OF ALTERNARIA TENUIS AND PHOMA HERBARUM. Morris A. Kaplan, M.D., Assistant Professor of Medicine, The Chicago Medical School, and Director of the Allergy Research Unit, The Chicago Medical School, and The Mount Sinai Medical Research Foundation, Chicago, Illinois; Homer E. Prince, M.D., Clinical Professor of Medicine, Baylor University College of Medicine, Houston, Texas; and Marie B. Morrow, Ph.D., Associate Professor of Botany and Mycologist, Plant Research Institute, The University of Texas, Austin, Texas.

SOME EXPERIENCES WITH A NONSPORULATING STRAIN OF ALTERNARIA TENUIS. John M. Sheldon, M.D., Professor of Internal Medicine; Kenneth P. Mathews, M.D., Associate Professor of Internal Medicine; James A. McLean, M.D., Instructor of Internal Medicine; and Florence McKay, Technician, Department of Allergy, University Hospital, University of Michigan, Ann Arbor, Michigan.

STUDIES IN ALLERGENICITY OF EXTRACTS PREPARED FROM SPORULATING AND NONSPORULATING ALTERNARIA TENUIS. Mary C. Johnson, B.A., Research Assistant, Division of Immunology, Department of Medicine, Washington University School of Medicine, St. Louis, Missouri; and Stanley F. Hampton, M.D., Assistant Professor of Clinical Medicine, Washington University School of Medicine, and Director of Allergy Clinic, St. Louis, Missouri.

PRECONVENTION SCIENTIFIC ASSEMBLY (CONT'D)

Pediatric Allergy

2:00 P.M. Sunday, Feb. 5, 1956
Starlight Roof

Moderator: Charles A. Janeway, M.D., Thomas Morgan Rotch Professor of Pediatrics, Harvard Medical School; Physician in Chief, Children's Hospital, Boston, Massachusetts. (By invitation.)

INTRODUCTION.—Dr. Janeway.

ECZEMA.—Drs. Derbes and Mueller.

ASTHMA.—Dr. Overall.

Intermission

STEROID THERAPY.—Dr. Thurston.

GAMMA GLOBULIN.—Drs. Bernton and Bowen.

*Presented under the joint sponsorship of the American Academy of Allergy and the Association of Allergists for Mycological Investigations, Inc.

Intermission

QUESTIONS AND ANSWERS.

Participants:

Harry S. Bernton, M.D., Clinical Specialist in Allergy, Allergens Section, Agricultural Research Service, U. S. Department of Agriculture; Professor Emeritus of Hygiene and Preventive Medicine, Georgetown University Schools of Medicine and Dentistry, Washington, D. C.

Ralph Bowen, M.D., Chief of Allergy, Texas Children's Hospital, Houston, Texas.

Vincent J. Derbes, M.D., Professor of Medicine (Dermatology), Tulane University School of Medicine, New Orleans, Louisiana.

Harry L. Mueller, M.D., Chief of Allergy Clinic, Children's Hospital, Boston, Massachusetts.

James C. Overall, M.D., Professor of Clinical Pediatrics and Chief of Pediatric Allergy, Vanderbilt University, Nashville, Tennessee.

Donald L. Thurston, M.D., Assistant Professor of Pediatrics, School of Medicine, Washington University; Assistant Physician, St. Louis Children's Hospital; Director of the Allergy Division of the St. Louis Children's Hospital and of the Pediatric Clinic of the Washington University Clinics, St. Louis, Missouri.

SCIENTIFIC PROGRAM

Monday, Feb. 6, 1956
Morning Session, 9:00 A.M.

HARRY L. ALEXANDER, M.D., Chairman

1. **Changes in White Blood Cell Morphology and Serum Protein Fractions in Induced and Spontaneous Hypersensitivity States** (Abstract No. 16). Dorothy Ley, M.D., and J. D. L. Fitzgerald, M.D., Toronto, Canada.
2. **Isolation of Skin-Sensitizing Antibodies From Nonsensitizing Whole Serums of Humans and Immunized Rabbits** (Abstract No. 1). Frederick Aladjem, Ph.D., Walter R. MacLaren, M.D., Dan E. Campbell, Ph.D., and Wilton E. Vannier, M.D., Pasadena, California.
3. **A Comparative Study of Spontaneous and Induced Skin-Sensitizing Antibodies: Electrophorectic and Chemical Analyses** (Abstract No. 6). Robert A. Cooke, M.D., Arthur Menzel, M.D., Phyllis Myers, A.B., Joseph Skaggs, M.D., and Helen Zeman, M.D., New York, New York.

10:00 A.M. **Address by Charles A. Janeway, M.D., Thomas Morgan Rotch Professor of Pediatrics, Harvard University Medical School; Physician in Chief, Children's Hospital, Boston, Massachusetts. "The Gamma Globulins." (Sponsored through a grant by CIBA Pharmaceutical Products, Inc.)**

10:45-11:20. **Intermission to Register With Exhibitors.**

4. **Observations of Plasma 17-Hydroxycorticosteroid Levels in Allergic Children** (Abstract No. 27). Sheldon C. Siegel, M.D., Victor Birnberg, M.D., and Vincent C. Kelley, M.D., Los Angeles, California.
5. **Experimental Arterial Sensitization. Studies on the Specificity of "Shock Tissue"** (Abstract No. 5). Sheldon G. Cohen, M.D., Daniel S. Dzury, B.S., and John W. Walsh, M.D., Wilkes-Barre, Pennsylvania.
6. **Ragweed Pollen Desensitization in Hay Fever Relationship to Skin Test Reaction, Dosage, and Clinical Results** (Abstract No. 33). Louis Tuft, M.D., and V. Muriel Heck, M.T., Philadelphia, Pennsylvania.

12:00-2:00 P.M. **Luncheon Intermission**

Afternoon Session, 2:00 P.M.

ROBERT A. CHOBOT, M.D., Chairman

2 P.M. **Address by Herman N. Eisen, M.D., Professor of Medicine (Dermatology), Washington University School of Medicine, St. Louis, Missouri. "Some Mechanisms Involved in Allergic Contact Dermatitis." (Sponsored through a grant by CIBA Pharmaceutical Products, Inc.)**

7. **Pollen Formalinized Tannate in Desensitization Treatment** (Abstract No. 19). Hyman L. Naterman, M.D., Boston, Massachusetts.
8. **Treatment of Ragweed Hay Fever and Asthma With an Electrostatic Air Cleaning Device Combined With an Air Conditioner** (Abstract No. 20). Harold S. Novey, M.D., Walter S. Burrage, M.D., and Joseph I. Engler, M.D., Boston, Massachusetts.
9. **Variations in Ragweed Pollen Exposure of Individuals in a Metropolitan Area** (Abstract No. 14). Eloise W. Kailin, M.D., Washington, D. C.

3:45-4:10. **Intermission to Register With Exhibitors.**

10. **The Importance of Insect Antigens in Respiratory Allergy** (Abstract No. 8). Alan R. Feinberg, M.D., Samuel M. Feinberg, M.D., and Carlos Benaim-Pinto, M.D., Chicago, Illinois.
11. **Antigenic Relationships Between Honey Bees, Wasps, Yellow Jackets and Yellow and Black Hornets** (Abstract No. 29). Robert A. Stier, M.D., and Edward L. Foubert, Ph.D., Spokane, Washington.

Report of the Committee on Drugs, Research Council of the American Academy of Allergy. Sidney Friedlaender, M.D., Chairman.

Tuesday, Feb. 7, 1956
Morning Session, 9:00 A.M.

WILLIAM B. STEEN, M.D., Chairman

9:00-10:30. **Panel Discussion.**

"RECENT DEVELOPMENTS IN THE TREATMENT OF ALLERGIC DISEASES WITH STEROID HORMONES." Bram Rose, M.D., Moderator.

a. **Meticorten and Meticortelone in the Treatment of Various Allergic Conditions** (Abstract No. 2). Carl E. Arbesman, M.D., and Robert J. Ehrenreich, M.D., Buffalo, New York.
b. **The Use of Prednisolone (Sterane) in the Treatment of Severe Allergic Diseases** (Abstract No. 13). Thomas G. Johnston, M.D., and Alan G. Cazort, M.D., Little Rock, Arkansas.
c. **Meticorten (Prednisone) in the Treatment of Bronchial Asthma; A Clinical Study on 50 Patients** (Abstract No. 30). Samuel J. Taub, M.D., Morris A. Kaplan, M.D., and Abe A. Aaronson, M.D., Chicago, Illinois.
d. **Prednisone and Prednisolone in Bronchial Asthma and Perennial Allergic Rhinitis** (Abstract No. 4). Earl B. Brown, M.D., and Thomas Seideman, M.D., New York, New York.
e. **Experience With the Use of Four Currently Available Steroids in Allergic Diseases** (Abstract No. 25). Irving W. Schiller, M.D., Francis C. Lowell, M.D., William Franklin, M.D., and Alan L. Michelson, M.D., Boston, Massachusetts.
f. **Experiences With Metacortandracine and Metacortandralone in Bronchial Asthma** (Abstract No. 26). John M. Sheldon, M.D., Kenneth P. Matthews, M.D., and James A. McLean, M.D., Ann Arbor, Michigan.
g. **Metabolic Studies on Meticorten in Cases of Allergy** (Abstract No. 23). Bram Rose, M.D., and K. R. MacKenzie, M.D., Montreal, Canada.
h. **Autopsy Findings in Four Patients Under Prolonged Treatment With Steroids for**

Allergic Disease (Abstract No. 9). William Franklin, M.D., Francis C. Lowell, M.D., Irving W. Schiller, M.D., and Sheldon C. Sommers, M.D., Boston, Massachusetts.

10:30-10:50. **Intermission to Register With Exhibitors.**

10:50. **Address by Carl V. Moore, M.D., Busch Professor of Medicine, Chairman of Department of Medicine, Washington University School of Medicine; Physician-in-Chief, Barnes Hospital, St. Louis, Missouri. "Immunohematology." (Sponsored through a grant by CIBA Pharmaceutical Products, Inc.)**

12. **Serum Proteins in Diseases of Hypersensitivity Including Collagen Disease** (Abstract No. 22). Bram Rose, M.D., H. Hollinger, M.D., and A. H. Sehon, M.D., Montreal, Canada.

11:50 A.M.-12:05 P.M. **An Answer to a Question.** Lawrence J. Halpin, M.D., President, American College of Allergists.

12:15-2:00. **President's Luncheon, Starlight Roof. For Members, Wives, and Guests.**

Afternoon Session, 2:00 P.M.

WALTER S. BURRAGE, M.D., Chairman

2 P.M. **Address by Sidney Raffel, M.D., Professor of Bacteriology, Stanford University, Stanford, California. "Bacterial Hypersensitivity." (Sponsored through a grant by CIBA Pharmaceutical Products, Inc.)**

13. **The Role of CRP in Allergic Inflammation; The Relationship Between the Acute Phase Response and Antibody Titer** (Abstract No. 15). M. A. Kaplan, M.D., Arnaldo Libretti, M.D., and Betty Goldman, B.S., Chicago, Illinois.

3:05-3:30. **Intermission to Register With Exhibitors.**

14. **Precipitating Antibodies in Human Sera Against Material Derived From Staphylococci** (Abstract No. 3). Sam M. Beiser, Ph.D., Murray Dworetzky, M.D., K. Marilyn Smart, M.A., and Horace S. Baldwin, M.D., New York, New York.

15. **Protective Action of Gamma Globulin Against the Anaphylactoid Effects of a Staphylococcal Bacterial Fraction, as Tested Upon Isolated Guinea Pig Ileum** (Abstract No. 7). Murray Dworetzky, M.D., Sam M. Beiser, Ph.D., K. Marilyn Smart, M.A., and Horace S. Baldwin, M.D., New York, New York.

16. **Infectious Asthma and Intrafamilial Contagion** (Abstract No. 21). Samuel J. Prigal, M.D., New York, New York.

4:30. **Annual Business Meeting of the American Academy of Allergy, Starlight Roof.**

Wednesday, Feb. 8, 1956
Morning Session, 9:00 A.M.

CARL E. ARBESMAN, M.D., Chairman

17. **Eye Tests With Inhalant Allergens—Their Indications and Clinical Value** (Abstract No. 32). Louis Tuft, M.D., L. N. Ettelson, M.D., and Charles Krueger, B.S., Philadelphia, Pennsylvania.

18. **Blood Protein Abnormalities in Asthmatic Children** (Abstract No. 31). Harold S. Tuft, M.D., Denver, Colorado.

19. **Foreign Body in the Lung as the Cause of Asthma** (Abstract No. 11). James Holman, M.D., Dallas, Texas.

10:00. **Address by Irvin Kerlan, M.D., Associate Medical Director, Food and Drug Administration, Washington, D. C. "Foods, Drugs, Cosmetics and the Allergist." (Sponsored through a grant by CIBA Pharmaceutical Products, Inc.)**

10:30-11:00. Intermission to Register With Exhibitors.

20. **Transient Myxedema Produced by Prolonged Ingestion of Saturated Solution of Potassium Iodide** (Abstract No. 28). Joseph T. Skaggs, M.D., New York, New York.

21. **The Differentiation Between Bronchial Asthma and Chronic Obstructive Emphysema** (Abstract No. 17). Francis C. Lowell, M.D., William Franklin, M.D., Irving W. Schiller, M.D., and Alan L. Michelson, M.D., Boston, Massachusetts.

22. **A Study of Allergic Disease in Emotionally Disturbed Children** (Abstract No. 10). M. Coleman Harris, M.D., San Francisco, and Norman Shure, M.D., Los Angeles, California.

23. **The Use of N-(2-3, 4 Methylenedioxyphenyl) Isopropyl in Bronchial Asthma** (Abstract No. 12). Irving Itkin, M.D., Walter S. Burrage, M.D. (3), and John W. Irwin, M.D., Boston, Massachusetts.

24. **Allergic Diarrhea Due to Food Allergy** (Abstract No. 24). Albert H. Rowe, M.D., Albert Rowe, Jr., M.D., and Kahn Uyeyama, M.D., San Francisco, California.

25. **Allergotoxins and Antihistamines** (Abstract No. 18). M. H. Mothersill, M.D., Indianapolis, Indiana.

Common Weeds and Grasses (Colored Third Dimensional Slides). Mr. Hugh Graham, Dallas, Texas.

Abstracts of Papers to Be Presented

1. ISOLATION OF SKIN-SENSITIZING ANTIBODIES FROM NON-SENSITIZING WHOLE SERUMS OF HUMANS AND IMMUNIZED RABBITS

FREDERICK ALADJEM, PH.D., WALTER M. MACLAREN, M.D., DAN H. CAMPBELL, PH.D., AND WILTON E. VANNIER, M.D., PASADENA, CALIF.

Serums from clinically sensitive individuals often do not give positive passive transfer reactions. This is particularly noticeable in cases of food allergy. Also, serum from immunized rabbits, which may contain large amounts of precipitating antibodies, are often found to be inactive when tested by the passive transfer reaction in man. It has been possible to separate skin sensitizing antibody fractions from such non-reacting whole serums by two methods: ammonium sulfate precipitation and starch electrophoresis.

Serum studies were obtained from clinically egg-sensitive humans and from rabbits immunized against bovine serum albumin, ovalbumin, and beta lactoglobulin. Recipients were passively sensitized with unfractionated serum and with serum fractions containing about 0.1 per cent protein. Three to seven days later, the prepared sites were tested with 1 per cent antigen. The results were read 15 to 20 minutes later. It was found that the skin sensitizing activity of human serum was associated with material not precipitable with half-saturated ammonium sulfate but precipitable with 70 per cent saturated ammonium sulfate at pH 5.6. Skin-sensitizing material from rabbit serum was usually associated with material precipitable between 33 and 55 per cent saturated ammonium sulfate. Skin-sensitizing material could also be separated from whole human and rabbit serums by starch electrophoresis. The precipitating antibodies from rabbit serums were usually found to be in different fractions than the skin sensitizing material. The distribution of these antibodies on starch and the possible role of blocking antibodies will be discussed.

2. METICORTEN AND METICORTELONE IN THE TREATMENT OF VARIOUS ALLERGIC CONDITIONS

CARL E. ARBESMAN, M.D., AND ROBERT J. EHRENREICH, M.D., BUFFALO, N. Y.

In a preliminary report, the authors showed that Meticorten was at least as beneficial in the relief of symptoms in bronchial asthma as were the older steroids, cortisone and hydrocortisone. The effective dosage of Meticorten was approximately one-fourth that of hydrocortisone and one-fifth that of cortisone. In addition, it was shown that Meticorten had very little effect on body weight, blood pressure, or sodium retention.

This presentation is a continuation and enlargement of our previous studies. A total of seventy-eight patients having 108 different allergic disorders were treated with either Meticorten or Meticortelone at different times. Patients were transferred from Meticorten to Meticortelone, dose for dose, as soon as the latter was available for study. All subsequent patients requiring steroids were placed on Meticortelone. The relative effectiveness and dosage of these two new steroids in the same patients will be presented.

Thirty-six of these seventy-eight patients had had some other forms of steroid prior to this study. Here again a comparison between the effectiveness of these older steroids (ACTH, cortisone, and hydrocortisone) to either Meticorten or Meticortelone will be discussed. The patients were switched from a maintenance dosage of cortisone or hydrocortisone to either Meticorten or Meticortelone, tablet for tablet. In other words, for each 25 mg. tablet of cortisone or 20 mg. tablet of hydrocortisone, one 5 mg. tablet of either Meticorten or Meticortelone was given four times daily. The dosage was gradually lowered until the minimum amount which could give adequate relief was obtained. This varied from individual to individual and sometimes in the same patient at different times. However, the average maintenance dosage varied between 5 and 10 mg. daily.

In this group of patients, sixty-two had bronchial asthma, fifteen had perennial allergic rhinitis, thirteen had seasonal ragweed hay fever, eight had nasal polyps, six had atopic dermatitis, two had urticaria, one had contact dermatitis, and one had G-I allergy. None of these patients who had obtained relief from the usual orthodox methods of treatment, such as hyposensitization, antihistamines, bronchodilators, and allergic disorders to the new steroids Meticorten and Meticortelone will be presented. Likewise, the side effects and relative effectiveness will be compared.

3. PRECIPITATING ANTIBODIES IN HUMAN SERA AGAINST MATERIALS DERIVED FROM STAPHYLOCOCCI

SAM M. BEISER, PH.D., MURRAY DWORETZKY, M.D., MARILYN SMART, M.A., AND HORACE S. BALDWIN, M.D., NEW YORK, N. Y.

As part of a broad investigation of the relation of staphylococci to allergic diseases, we have studied human sera for the presence of antibodies to materials derived from various strains of staphylococci.

Gamma globulin, prepared from pooled normal human sera and individual sera obtained from allergic and nonallergic persons, was studied. Culture filtrates, as well as extracts prepared from washed, ground bacterial cells, were used as antigens. In addition, chemical fractions of the culture filtrates and the bacterial extracts were studied.

The agar precipitin method of Ouchterlony was used throughout. In this procedure antiserum and antigen diffuse toward each other in a semisolid agar medium in a Petri plate. If the antiserum contains antibodies to

the antigen, a line of precipitate forms between the two. The number of lines formed is usually indicative of the number of antigen-antibody systems involved. Our results indicate that human serum contains antibodies to materials derived from *Staphylococcus aureus,* but no antibodies were demonstrable against materials prepared from *Staphylococcus albus.* Antibodies were found against culture filtrates and cell extracts. It is of interest that antibodies were formed against more constituents of the culture filtrate than were formed against materials in the cell extracts. Antibodies have been demonstrated to at least six components of the culture filtrate.

The correlation between the presence of antibodies in individual sera and allergic manifestations will be presented and discussed.

4. THE USE OF PREDNISONE AND PREDNISOLONE IN BRONCHIAL ASTHMA AND PERENNIAL ALLERGIC RHINITIS

EARL B. BROWN, M.D., AND THOMAS SEIDEMAN, M.D., NEW YORK, N. Y.

Approximately seventy-five patients were given prednisone and prednisolone for the treatment of bronchial asthma and perennial allergic rhinitis. In some cases the drug was given continuously for many months, and in others it was given intermittently, depending upon the patient's symptoms. All patients were ambulatory. A record was kept of weight and blood pressure. Indicated blood and urine chemistries were done. Patient's response to the drug was charted and all side reactions were enumerated. In this paper will be discussed the management of the patients with the accumulated data, as well as some illustrative case histories.

5. EXPERIMENTAL ARTERIAL SENSITIZATION; STUDIES ON THE SPECIFICITY OF "SHOCK TISSUE"

SHELDON G. COHEN, M.D., DANIEL S. DZURY, B.S., AND JOHN W. WALSH, M.D., WILKES-BARRE, PA.

Previous studies of the author have demonstrated that experimentally produced lesions of periarteritis and panarteritis are a function of the in vivo antigen-antibody reaction in the rabbit. Passive sensitization with high titer homologous antiserum and subsequent challenge with subclinical anaphylactic doses of specific antigen administered intravenously favors the production of these pulmonary vascular lesions. This described technique has been utilized to study the specificity of the pulmonary vasculature in its participation in sensitization reactions as "shock tissue" as noted in active and passive sensitization experiments. The question is raised as to whether this immunologic phenomenon may not be related to the fact that the pulmonary tissue is the first organ supplied by circulating antibody and antigen after its intravenous introduction. This possibility is further evaluated by similar experimental studies employing passive sensitization with homologous high-titer horse antiserum and subsequent challenge with dilute horse serum via varying routes of administration designed to initially expose those organs thus directly supplied with antibody and antigen: (1) ear veins through the heart to pulmonary tissue, (2) the exposed cannulated carotid artery to cerebral tissue, (3) intraperitoneally into the portal system to hepatic tissue. Results of microscopic study of tissue sections of the heart, lung, brains, and liver are presented in an attempt to correlate the presence of vascular lesions with the route of administration.

6. A COMPARATIVE STUDY OF SPONTANEOUS AND INDUCED SKIN-SENSITIZING ANTIBODIES: ELECTROPHORETIC AND CHEMICAL ANALYSES

ROBERT A. COOKE, M.D., ARTHUR MENZEL, M.D., PHYLLIS MEYER, A.B., JOSEPH SKAGGS, M.D., AND HELEN ZEMEN, M.D., NEW YORK, N. Y.

Chemical and electrophoretic studies of skin-sensitizing human sera indicate differences in the nature and properties of the skin-sensitizing antibodies in spontaneously acquired sensitization, as exemplified by ragweed hay fever, and artificially induced sensitization, exemplified by serum sickness.

These differences suggest that the mechanism of sensitization may be somewhat different in these two cases.

The skin-sensitizing antibody in serum sickness, like precipitin, is mainly in the gamma globulin, while the skin-sensitizing antibody in hay fever appears in various different fractions of the globulin, depending on the procedure followed.

7. PROTECTIVE ACTION OF GAMMA GLOBULIN AGAINST THE ANAPHYLACTOID EFFECTS OF A STAPHYLOCOCCAL BACTERIAL FRACTION, AS TESTED UPON ISOLATED GUINEA PIG ILEUM

MURRAY DWORETZKY, M.D., SAM M. BEISER, PH.D., K. MARILYN SMART, M.A., AND HORACE S. BALDWIN, M.D., NEW YORK, N. Y.

The present work is part of a study of the role of staphylococcus in bacterial hypersensitivity.

Previous investigations in this laboratory have demonstrated that a fraction, called Fraction A, derived from washed, ground pathogenic hemolytic staphylococcus organisms had an anaphylactoid effect upon the isolated segment of guinea pig ileum. This fraction is heat stable, and is probably a nucleoprotein, and its effect upon the isolated guinea pig ileum was shown to be strikingly different from that caused by heat-labile pathogenic staphylococcus culture filtrate.

The protective effect of sera and protein fractions from sera of various species against the anaphylactoid reaction of Fraction A was studied, utilizing isolated segments of guinea pig ileum, suspended in a 5.0 ml. bath of Tyrode solution maintained at 37° C. Sera or serum protein fractions were first placed in the bath, and thirty seconds to one minute later than the Fraction was added. Protection was demonstrated first by absence of contraction to Fraction A, and then substantiated by the production of an anaphylactoid contraction to Fraction A again added to the bath after completely washing out the serum or protein fraction.

Sera from normal horse, rabbit, guinea pig, bovine and human sources were all shown to have this protective effect against Fraction A. This protection was found present in the gamma globulin fraction and absent in all other serum protein fractions. No other materials studied, including glycerin, egg white, egg yolk or cobra venom, prevented the anaphylactoid effect of Fraction A.

Serum or gamma globulin failed to protect the isolated ileum against the effects of any of the other myotonic agents studied, including pathogenic staphylococcal culture filtrate, cobra venom, and histamine. In addition, anaphylaxis, produced in segments of ileum from guinea pigs sensitized to various proteins unrelated to Fraction A, was not prevented or altered by preceding addition to the bath of serum or gamma globulin.

It is felt that this protective agent in the gamma globulin fraction is most likely an antibody or other protective substance. Whether it is a naturally occurring or acquired antibody is under consideration.

8. THE IMPORTANCE OF INSECT ANTIGENS IN RESPIRATORY ALLERGY

ALAN R. FEINBERG, M.D., SAMUEL M. FEINBERG, M.D., AND CARLOS BENAIM-PINTO, M.D., CHICAGO, ILL.

Allergy to insects has been regarded more or less as a medical curiosity. Reports have dealt mainly with severe or fatal reaction to the stings of bees or similar insects or they have been concerned with special occupational exposures or seasonal manifestations of short duration in localized areas.

We have reason to suspect that allergy to insect antigens is much more widespread than customarily believed. Our suspicions have been based on several considerations. Using a number of insect antigens we have obtained unquestioned and strongly positive skin tests mainly by the scratch method, in a fairly large series of patients. The immunologic significance of these reactions has been strengthened by the tendency of many insect antigens to cross react. Some of these patients had seasonal hay fever or asthma: others had perennial symptoms or perennial symptoms with seasonal exacerbations. A number of patients have had desensitization treatment with insect antigens with encouraging results. Some of these patients had had desensitization with other inhalants, seasonal or perennial, without benefit. The incidence of insect life in the soil, as recorded by the U. S. Department of Agriculture, is tremendous. We believe it is possible that a major source of insect antigens may well be soil dust.

The data on more than 100 patients with insect allergy will be presented. This will include the cross reactivity, the presence of other allergy, and the results of desensitization in some of them. Some immunologic studies on precipitin, gel diffusion, and reagin neutralization will also be presented.

9. AUTOPSY FINDINGS IN FOUR PATIENTS UNDER PROLONGED TREATMENT WITH STEROIDS FOR ALLERGIC DISEASE

WILLIAM FRANKLIN, M.D., FRANCIS C. LOWELL, M.D., IRVING W. SCHILLER, M.D., AND SHELDON C. SOMMERS, M.D., BOSTON, MASS.

In the course of following a considerable number of patients receiving intensive steroid therapy, we have had four deaths. The first was a woman of 68 who, having been successfully treated for three years, finally developed intractable asthma in spite of very intensive steroid therapy and what appeared to be cardiac failure. A 59-year-old man with asthma and emphysema, having been fairly successfully treated for five years, developed recurring pancreatitis. A 45-year-old man with progressive pulmonary fibrosis developed osteoporosis and collapse of vertebrae during treatment over a four-year period, accompanied by apparent progression of the pulmonary lesion. A 55-year-old man with a severe chronic dermatitis developed severe and widespread furunculosis and died with staphylococcal septicemia after a year of steroid therapy.

The clinical course and autopsy findings in these patients will be presented and discussed with particular attention to the status of the adrenal and pituitary glands.

10. A STUDY OF ALLERGIC DISEASE IN EMOTIONALLY DISTURBED CHILDREN

M. Coleman Harris, M.D., San Francisco, and Norman Shure, M.D., Los Angeles, Calif.

In recent years much emphasis has been placed upon the importance of the emotional factor in the production of allergic disease in children. Specific emotional patterns have been suggested for bronchial asthma, eczema, and urticaria. Mainly, the investigators have used comparatively small groups of children diagnosed as having had bronchial asthma, eczema, or urticaria. The authors have reversed the process and have studied large numbers of emotionally disturbed children in two large cities, San Francisco and Los Angeles, to determine (1) the frequency with which these diseases occur and (2) if any specific pattern can actually be demonstrated. Bronchial asthma was emphasized in the studies, since it is in this disease that a specific emotional pattern most frequently is stressed. Results of the survey and conclusions of the authors are presented.

11. FOREIGN BODY IN THE LUNG AS THE CAUSE OF ASTHMA

James Holman, M.D., Dallas, Texas

Two case presentations of an asthmatic syndrome precipitated by a foreign body in the bronchus are presented. The unusual features of the first case are as follows:

(1) Absence of demonstration of a chicken bone in the lung field on numerous x-ray examinations.

(2) Inability of the bronchoscopist to demonstrate a foreign body in the bronchus on two separate examinations, and subsequent finding of the chicken bone on the third bronchoscopic examination, approximately two years after the onset of symptoms. Demonstration of the bone was made possible by the patient having developed pneumonia in the left lower lobe twenty months after the onset of symptoms.

(3) The patient had bilateral, evanescent râles typical of bronchial asthma over the two-year period. There were never any localized signs prior to the pneumonia which occurred twenty months after the foreign body was first lodged in the bronchus.

The second case presentation is that of a 20-month-old infant who developed a sudden attack of asthma.

(1) There was a questionable history of the baby's having swallowed a foreign body.

(2) The x-ray film did not demonstrate a foreign body.

(3) This patient had bilateral râles that were constant and not evanescent.

(4) This patient came from an asthmatic family and was thought to have developed asthma on two occasions prior to swallowing tin foil.

(5) It was felt wise to bronchoscope the child in the face of a negative x-ray examination for two reasons:

(a) Because of inability to alleviate the symptoms by administration of drugs

(b) Because of the constant symptoms over a period of two months with no remission in the dyspnea.

At the time of bronchoscopy the tin foil was found in the right main stem bronchus. X-ray film slides will be shown.

12. THE USE OF N-(2-(3, 4 METHYLENEDIOXYPHENYL) ISOPROPYL IN BRONCHIAL ASTHMA

IRVING ITKIN, M.D., WALTER S. BURRAGE, M.D., AND JOHN W. IRWIN, M.D., BOSTON, MASS.

N-(2-(3, 4 methylenedioxyphenyl) isopropyl is a bronchodilator. For the past year this compound has been used to suppress symptoms of asthma of both extrinsic and undetermined origin. A double blindfold test was used to compare the oral preparation of this agent with that of ephedrine. Another double blindfold test with the above N-substituted arterenol derivative and with epinephrine was tried when both agents were given by inhalation. Comparisons were based on subjective and objective observations. Timed vital capacity and maximum breathing capacity before, during, and after therapy have been included.

Effectiveness of this compound in symptomatic relief of asthma, dosage schedules, and side reactions will be discussed.

13. THE USE OF PREDNISOLONE (STERANE) IN THE TREATMENT OF SEVERE ALLERGIC DISEASES

THOMAS G. JOHNSTON, M.D., AND ALAN G. CAZORT, M.D., LITTLE ROCK, ARK.

The purpose of this presentation is to relate our experiences with the use of prednisolone (Sterane) in the treatment of severe allergic diseases.

Over 100 patients with various allergies such as ragweed hay fever, bronchial asthma, atopic dermatitis, contact dermatitis, drug eruption, etc., have been treated with prednisolone (Sterane) since August, 1955.

The usual dosage administered was a total of 150 mg., taken over twelve days, giving 20 mg. daily for three days, 15 mg. daily for three days, 10 mg. daily for three days, and 5 mg. daily for three days in divided doses. Several patients were given two to four courses. An antacid was routinely given between meals and at bedtime. An attempt will be made to compare the effectiveness of prednisolone (Sterane) with that of cortisone, hydrocortisone, and prednisone when possible. It is our opinion that prednisolone is the drug of choice among these four medications.

The results, as with any new medication, are difficult to evaluate. However, the majority of the patients thought prednisolone (Sterane) was the best medication of its type because of its effectiveness, promptness of action, ease of administration, lack of the need of salt restriction, and its low incidence of side reactions.

Side reactions were chiefly gastrointestinal, although there were rare cases of insomnia, constipation, increased appetite, nervousness, headache, and acneform eruptions. The gastrointestinal symptoms were chiefly "gas" and "indigestion," with nausea and vomiting occurring in two patients. In only two patients was it necessary to discontinue the medication before the completion of the prescribed course.

It is our opinion that prednisolone (Sterane) has a definite place in our drug armamentarium against severe allergic diseases.

We still consider all these drugs as emergency medications for short periods of use to allay unusually severe symptoms.

14. VARIATIONS IN RAGWEED POLLEN EXPOSURE OF INDIVIDUALS IN A METROPOLITAN AREA

ELOISE W. KAILIN, M.D., WASHINGTON, D. C.

Pollen slides were pinned on the left lapels of ninety-five individuals in Washington, D. C., simultaneously to gain some idea of the variability of individual exposure to ragweed pollen over a twenty-four-hour period. Slides were protected with coarse wire net. At night they were placed on the bureau. These pollen counts were studied with respect to area of residence and business, time spent in automobiles, outdoors, in air-filtered rooms, and indoors with windows open and with windows closed. The exposure was made on a sunny working day. Thirty-two husband-wife comparisons are available.

The average catch on a 3.6 sq. cm. slide area was 11.6 grains, varying from less than 1 to 49 grains. Standard shelter slide exposures at different points in the city were 54, 87, and 191 ragweed grains per 3.6 sq. cm. slide area for the same period of time. Housewives tended to higher counts than their (office worker) husbands, and spent more time outdoors. Number of hours spent in an automobile had very little effect on pollen exposure. Time spent in air-filtered rooms or in rooms with closed windows related to a smaller pollen catch on the average. However, four persons who spent fifteen or more hours in closed or air-filtered rooms showed counts of 10, less than 1, 16, and 4 grains, respectively. The area of the city having the lowest outdoor standard count showed lower average counts for the group residing there.

It is concluded that variability in ragweed exposure is extremely wide and often unpredictable for the individual.

15. THE ROLE OF CRP IN ALLERGIC INFLAMMATION; THE RELATIONSHIP BETWEEN THE ACUTE PHASE RESPONSE AND ANTIBODY TITER

M. A. KAPLAN, M.D., ARNALDO LIBRETTI, M.D., AND BETTY GOLDMAN, B.S., CHICAGO, ILL.

A survey has been performed to determine the relationships existing between the presence of CRP in the blood stream of patients with an acute inflammatory process, and the production and titer of specific antibodies.

The present study is concerned with:

(a) Patients who allowed tonsillar or pharyngeal exudate and who were found to have ten or more colonies of typable group A (Lancefield) streptococci on throat culture. Patients with suppurative complications during their illness were discarded.

(b) Patients showing clinical evidences of frank rheumatic activity (according to the criteria of Jones).

The patients were also subdivided according to the therapeutic procedures.

Throat cultures, C-reactive protein (CRP), and antistreptolysis-O (ASO) titers were determined weekly for several weeks.

CRP in amounts varying from 0.003 to 0.140 mg. per cubic centimeter of serum was found to be present in 87 per cent of the patients in whom throat culture showed pathologic growth of typable group A streptococci.

A close relationship was found between the amount of CRP present in the blood at the time of tonsillar or pharyngeal exudate and the subsequent rise of the ASO titer.

CRP was always present in the patients with acute rheumatic fever, and its variations were a very sensitive indicator of the rheumatic activity. The ASO titer, during this period, was very inconsistent. No significant rise in ASO titer was observed until the acute phase began to subside. The rise in ASO titer at this time semed to be correlated with a decrease in the amount of CRP present in the blood.

The possible interpretations will be discussed.

16. CHANGES IN WHITE BLOOD CELL MORPHOLOGY AND SERUM PROTEIN FRACTIONS IN INDUCED AND SPONTANEOUS HYPERSENSITIVITY STATES

DOROTHY LEY, M.D., AND J. D. L. FITZGERALD, M.D., TORONTO, CANADA

This study is concerned with the white blood cellular response to acute hypersensitivity reactions and the associated changes in serum protein patterns. Although eosinophilia is often associated with allergic reactions, recent workers have focused attention on the plasma cell and the lymphocyte as the cells of major importance in both the local and peripheral pathology of hypersensitivity states, and have emphasized the concomitant changes in serum globulins. With these facts in mind, we became interested in the lymphocyte-plasma cell series, its relationship to serum protein, and the changes produced in both systems by allergic reactions.

We have investigated, therefore, the peripheral blood morphology and serum protein fractions (sodium sulfate precipitation method) of patients seen because of acute reactions to tetanus antiserum. When the reaction was sufficiently severe to warrant hospitalization and adrenal hormone therapy, these determinations were repeated after the clinical symptoms had subsided. Because a relatively consistent protein response by this method was demonstrated, we are now comparing the patterns obtained by routine salting out with those found on filter paper electrophoresis. Conversion of the stained protein strips to absolute values is obtained by employing an electronic scanning device.

As well as changes in this induced allergic state, we are observing the white cell morphology and protein patterns of "hypersensitivity states," including "collagen diseases," glomerulonephritis, acute rheumatic fever, and spontaneously occurring allergic diseases such as hay fever, asthma, etc. These we propose to use as comparative groups in the interpretation of our results. A group of nonallergic controls will be included.

17. THE DIFFERENTIATION BETWEEN BRONCHIAL ASTHMA AND CHRONIC OBSTRUCTIVE EMPHYSEMA

FRANCIS C. LOWELL, M.D., WILLIAM FRANKLIN, M.D., IRVING W. SCHILLER, M.D., AND ALAN L. MICHELSON, M.D., BOSTON, MASS.

The increasing awareness of the physiologic changes which characterize asthma and emphysema has led to increasing efforts to distinguish clearly between the two states. Although there are striking similarities in the degree

and the nature of the functional pulmonary impairment, there are also important differences in the mode of onset, the manner of progression, the degree of reversibility, whether spontaneously or therapeutically induced, and more speculatively, the pathogenesis. To illustrate these points, the clinical features and the studies of pulmonary function in a group of more than thirty patients with clear-cut progressive obstructive pulmonary emphysema are compared with those of patients with bronchial asthma.

Our observations are consistent with the hypothesis that both diseases arise from inhalation of noxious particles, those giving rise to asthma being relatively large (pollens, certain dusts, and mold spores) and those causing emphysema being minute (smoke and certain industrial dusts). In keeping with this hypothesis was the finding that all the patients in the group with emphysema had been heavy smokers.

18. ALLERGOTOXINS AND ANTIHISTAMINICS

M. H. Mothersill, M.D., Indianapolis, Ind.

Dragstedt (*Ann. New York Acad. Sc.* **50**: 1039, 1950) reviewed the evidence which indicates that the site of an immunologic allergic reaction may be within the tissue cells and that the interaction of allergen and reagin may produce sufficient cellular injury to release the cell contents. Among the substances which might be released are histamine, heparin, acetylcholine, lysocithin, potassium, and carious intracellular enzymes. In so far as any of these substances is toxic, when it becomes extracellular, it could be an allergotoxin.

Many of the symptoms of anaphylactic shock and of localized allergic reactions are attributable to histamine. Heparin probably plays a role in the blood incoagulability which is seen in anaphylactic shock. The degree of importance of other cellular contents has not yet been evaluated.

Wells (*Ann. New York Acad. Sc.* **50**: 1202, 1950) stated that the antihistaminic compounds probably act by competitive inhibition—in other words, in a tissue. If histamine wins out in the competition, it exerts its typical physiologic action. If the antagonist wins out, the symptoms of histamine toxicity are prevented; this is desirable, provided that the toxicity and the side effects of the required dose of the antagonist are less harmful than those of histamine.

Assuming that the mode of action of antihistaminic compounds is competitive inhibition, then the greater the concentration of free histamine in the shock tissue the greater must be the concentration of antihistamine potency required to prevent symptoms.

The amount of release histamine would be proportional to two variables: (1) the degree of hypersensitivity of the tissue and (2) the amount of allergen reaching that tissue.

During the last seven years we have collected a considerable number of case histories of hay fever patients who took antihistamine drugs and have attempted to correlate the relationship of the pollen count, the degree of sensitivity, and the variation in the dose required to produce sufficiently prolonged relief. A few typical case reports are given.

19. POLLEN FORMALINIZED TANNATE IN DESENSITIZATION TREATMENT

Hyman L. Naterman, M.D., Boston, Mass.

Methods to slow absorption of injected antigens have included emulsification of pollen extract in oil with various adjuvants, addition of gelatin to pol-

len extract, hydrochloric acid precipitates of pollen extract suspended in water, and tannic acid precipitates suspended in water or in oil with adjuvants. The specific activity of the pollen antigens has not been affected by these measures.

Formalinization of antigens under controlled conditions is a common immunologic procedure which chemically alters but does not destroy antigenicity. Partially formalinized pollen tannates were used for several years to desensitize. In 1954 and 1955, patients were treated with fully formalinized pollen tannates suspended in water. This paper describes the preparation and use of pollen formalinized tannates and the clinical results in 355 individual pollen seasons. The usual maximum dose of 0.25 mg. protein nitrogen was reached in two to four injections. The average total number of injections per preseasonal treatment in 1954 and 1955, respectively, was: trees 5.6 and 5.7; grass, 6.7 and 5.9; ragweed, 8.8 and 6.1. The average number of injections in perennial treatment for ragweed in 1954 was 10.1 and in 1955, 6.5. By clinical impression the relief obtained was considered generally satisfactory.

It is believed the use of pollen formalinized tannates for desensitization will give clinical relief as great as or greater than the usual methods, with fewer injections and with greater safety.

20. TREATMENT OF RAGWEED HAY FEVER AND ASTHMA WITH AN ELECTROSTATIC AIR CLEANING DEVICE COMBINED WITH AN AIR CONDITIONER

HAROLD S. NOVEY, M.D., WALTER S. BURRAGE, M.D., AND JOSEPH I. ENGLER, M.D., BOSTON, MASS.

Electrostatic precipitators have long been available for removing pollen from rooms. Prolonged closing of a room for maximum efficiency of such a unit is, at times, most uncomfortable during hot weater. The cost of an air conditioner and a separate electrostatic precipitator often has been prohibitive for many patients. An electrostatic precipitator and an air cooling device have been placed into one cabinet.

A full description of the combined unit will be presented. These new units have been tested under laboratory conditions, in hospital rooms, and in patients' homes. In addition, the air conditioner alone has been compared with the combined unit for efficiency in pollen removal. In such tests, two methods have been used: (1) exposure of pollen slides in the rooms fitted with these devices and (2) the placing of patients with symptoms of ragweed asthma and hay fever in such rooms for varying periods during the ragweed season. Problems encountered as well as clinical results and pollen counts of the equipped rooms will be discussed.

21. INFECTIOUS ASTHMA AND INTRAFAMILIAL CONTAGION

SAMUEL J. PRIGAL, M.D., NEW YORK, N. Y.

Asthma is frequently associated with infection in the respiratory tract and, like all infections, has an element of contagion. This was corroborated by an investigation of eighty-two families in which one or more members had asthma associated with infection. Seventy-seven of these families were studied by bacteriologic methods. In forty-five families all members of the household were cultured; in the remainder (thirty-two) the patient and at least one other member of the family suspected of being a carrier were cul-

tured. In fifty families there was evidence suggestive of contagion. The more complete the family study, the more evidence was available for contagion.

In some families, where the hemolytic staphylococcus (coagulase-positive) was the dominant organism, cultures of the entire family were taken at intervals throughout the year and the identity of the staphylococci were checked by phage typing. This revealed the continued persistence of these organisms scattered throughout the family, producing a variety of infections and, in allergic individuals infectious asthma.

In one family, in which three members had asthma, the bacteriologic studies included the environment (dust, utensils, clothing, etc.) before and after moving into a new apartment. The identical organisms, as determined by phage typing, were brought along into the new apartment and disseminated into the environment, indicating a possible pathway for contagion.

The question is raised as to whether earlier statistical studies of asthma which strongly implicated heredity as the prime factor overlooked the role of infection and possible intrafamilial contagion.

22. SERUM PROTEINS IN DISEASES OF HYPERSENSITIVITY INCLUDING COLLAGEN DISEASE

BRAM ROSE, M.D., H. HOLLINGER, M.D., AND A. H. SEHON, M.D., MONTREAL, CANADA

During the past two years estimations of the proteins in serum have been made in a number of different disease states, including asthma, urticaria, periarteritis nodosa, penicillin reactions, serum sickness, erythema nodosum, and lupus erythematosus disseminata.

The determinations were made by both paper electrophoresis and free electrophoresis in a Tiselius apparatus (Spinco 3 channel).

Marked alterations have been noted in many of these conditions, particularly when the determinations are made at frequent intervals during the course of remissions and exacerbations and after the administration of steroids. The relation of these changes to the various underlying disease mechanisms will be discussed.

23. METABOLIC STUDIES ON METICORTEN IN CASES OF ALLERGY

BRAM ROSE, M.D., AND K. R. MACKENZIE, M.D., MONTREAL, CANADA

Balance studies were made on hospitalized cases of asthma and other allergic diseases. These included nitrogen electrolytes, vitamin C, serum proteins by free electrophoresis, cholesterol, NPN, blood sugar curves, homograms, and urinary steroid excretion. Respiratory function studies and x-rays of the gastrointestinal tract before and after treatment were also carried out. The patients were hospitalized for a control period of about seven to ten days, after which Meticorten was administered in a dose range of 30 mg. per day by mouth.

The clinical response of these patients was correlated with changes in respiratory function. In general, Meticorten produced a drop in eosinophiles, increase in urinary excretion of glucocorticoids, suppression of 17-ketosteroids, increase in urine volume, and vitamin C output. No alteration of electrolyte or nitrogen balance occurred. These findings will be discussed in relation to clinical evaluation of Meticorten and Meticortelone in a group of 120 cases of allergy, asthma, and hay fever in particular.

24. ALLERGIC DIARRHEA DUE TO FOOD ALLERGY

ALBERT H. ROWE, M.D., ALBERT ROWE, JR., M.D., AND KAHN UYEYAMA, M.D., SAN FRANCISCO, CALIF.

This paper reports diarrhea due to food allergy, observed and studied by us for many years. In patients suffering with this affliction, other causes of diarrhea, such as achylia, intestinal parasites, chronic ulcerative colitis, regional enteritis, and other less frequent causes, have been ruled out by laboratory and x-ray studies and by proctoscopy. The symptoms in a series of thirty relieved cases, with the occurrence of other manifestations of personal and familial allergy and the infrequency of skin reactions to foods and other allergens will be presented. Reports of individual patients in childhood, mid-adult life, and old age will be summarized. The laboratory findings and the usual negative x-ray findings which characterize these cases will be included. The method of diagnosis, treatment, and successful control will terminate the paper.

25. EXPERIENCE WITH THE USE OF FOUR CURRENTLY AVAILABLE STEROIDS IN ALLERGIC DISEASES

IRVING W. SCHILLER, M.D., FRANCIS C. LOWELL, M.D., WILLIAM FRANKLIN, M.D., AND ALAN L. MICHELSON, M.D., BOSTON, MASS.

A large group of patients who had been treated with cortisone and hydrocortisone were also treated with prednisone and prednisolone. All four agents have proved very effective in relieving the manifestations of allergic disease. The drugs exhibited marked differences in activity on a weight basis and showed some differences in certain side effects and time relationships. Prednisone and prednisolone were well tolerated and caused less rounding of the face and edema than cortisone or hydrocortisone. They appear to be especially advantageous in those patients with cardiac failure requiring therapy with these agents.

Some patients have received treatment with steroids for four years or more, and the problems arising in this group will also be discussed.

26. EXPERIENCES WITH METACORTANDRACINE AND METACORTANDRALONE IN BRONCHIAL ASTHMA

JOHN M. SHELDON, M.D., KENNETH P. MATHEWS, M.D., AND JAMES A. MCLEAN, M.D., ANN ARBOR, MICH.

We have had an opportunity to follow approximately seventy-five asthmatics on Metacortandracine and Metacortandralone over a period of one to ten months. The purpose of this paper will be to demonstrate some metabolic studies on these patients relative to sodium, water, potassium and 17-ketosteroid determinations. We will also illustrate by means of scatter charts the weekly progress as to body weight, blood pressure, vital capacity and circulating eosinophile determinations. The undesirable side effects observed in this group of patients will also be reported.

Observations at this time would justify the following conclusions: (1) These compounds have profound antiasthmatic properties; (2) sodium and water retention and potassium depletion have not been of clinical importance; (3) when these drugs are discontinued, most of the patients studied have had a recurrence of their asthma; (4) important side reactions, such as moon facies (substitution of Metacortandracine or Metacortandralone in selective in-

stances, however, may result in improvement of the Cushing's syndrome previously induced by other corticosteroids), sleeplessness, and varied gastrointestinal disturbances, are commonly seen with prolonged administration of these compounds; (5) administration of Metacortandracine and Metacortandralone entails many of the same metabolic hazards as other adrenal corticosteroids; and (6) Metacortandracine and Metacortandraline, in general, can be substituted one for the other in comparable dosage with similar clinical and metabolic effects.

27. OBSERVATIONS OF PLASMA 17-HYDROXYCORTICOSTEROID LEVELS IN ALLERGIC CHILDREN

SHELDON C. SIEGEL, M.D., VICTOR BIRNBERG, M.D., AND VINCENT C. KELLEY, M.D., LOS ANGELES, CALIF.

Over the past year, observations of plasma 17-hydroxycorticosteroid levels were made in a series of allergic and normal children ranging in age from 5 months to 13 years. The majority of the children had bronchial asthma. Blood specimens were drawn at four stages of severity of their diseases: asymptomatic, mild, moderate, and severe. In addition, blood specimens were drawn while some of the children were receiving variable amounts of prednisone and prednisolone. The plasma 17-hydroxycorticosteroid levels were determined by the method of Nelson and Samuels.

The results indicated that when the allergy was moderate or severe, the levels of 17-hydroxycorticosteroids were higher. The authors interpreted this finding of elevated plasma levels of the hormone as being due to the patient's adaptation to the stress of his disease. No statistical difference in 17-hydroxycorticosteroid levels was observed between the asymptomatic allergic children and those having mild symptoms. The levels of 17-hydroxycorticosteroids were found statistically to be directly correlated to the amount of prednisone and prednisolone given the patients, but no critical hormone level at which the majority of the patients became asymptomatic could be determined.

28. TRANSIENT MYXEDEMA PRODUCED BY PROLONGED INGESTION OF SATURATED SOLUTION OF POTASSIUM IODIDE

JOSEPH T. SKAGGS, M.D., NEW YORK, N. Y.

This is a case report on the development of a transient myxedema of a severe degree that apparently was based solely on the prolonged use of potassium iodide, in doses ranging from 15 to 30 drops of the saturated solution daily, for seven years. This patient, when 69 years of age in 1955, had been under more or less continuous observation since 1940, at which time he was treated for urticaria and angioedema with satisfactory results. He returned for treatment on account of the development of asthma in 1948. Diabetes was discovered in 1945. Asthma was diagnosed as intrinsic in type resulting from an infection with a primary hyperplastic sinusitis. Bilateral Caldwell-Luc operation was done and the treatment for the asthmatic condition was satisfactory. Because of recurring respiratory infection with mild asthmatic symptoms, he was maintained under perennial vaccine therapy. While under treatment in February, 1955, it was observed that he had been gaining a lot of weight and further investigation showed all clinical symptoms of a rapidly developing myxedema. There were constipation, inability to stay awake, and an increase of 20 pounds in weight in less than six months. He was found to have a bradycardia, a basal metabolic rate of –29, and it was then discovered

that he had been continuously taking potassium iodide originally prescribed seven years previously. Withdrawal of potassium iodide was followed by rapid spontaneous disappearance of myxedema.

29. ANTIGENIC RELATIONSHIPS BETWEEN HONEY BEES, WASPS, YELLOW JACKETS, AND YELLOW AND BLACK HORNETS

ROBERT A. STIER, M.D., AND EDWARD L. FOUBERT, PH.D., SPOKANE, WASH.

Newspaper stories each summer of deaths from stinging insects are liberally documented by reports in the medical literature of fatal and near-fatal anaphylactic reaction following such stings. Clinical experience and experimental work indicate that patients surviving these allergic reactions can be desensitized to gain protection from further reactions.

Desensitization with whole insect body has its advocates, as does also desensitization with venom alone, with one insect genus, with specific genera, and with multipotent antigens. There is some uniformity to the opinion that skin testing is not very helpful in detecting the insect to which the patient is sensitized. In an effort to answer some of these questions, the following experiments were carried out.

Antisera to honey bees, yellow hornets, yellow jackets, black hornets, and wasps were produced in rabbits by the use of preliminary subcutaneous injections of antigen in Freund's adjuvant followed by intraperitoneal injections of alum-precipitated antigen. Precipitin titers were run on all collected sera; gel diffusion studies and experiments on active anaphylaxis in guinea pigs, using homologous and heterologous sera were conducted. Various methods of extraction and purification of whole insect antigen were investigated in an effort to decrease the irritant quality of the crude extract and increase its specificity.

To date the experiments have indicated some common antigens shared by all these insects, the best relationships existing between yellow hornets, yellow jackets and black hornets.

30. METICORTEN (PREDNISONE) IN THE TREATMENT OF BRONCHIAL ASTHMA; A CLINICAL STUDY ON 50 PATIENTS

SAMUEL J. TAUB, M.D., MORRIS A. KAPLAN, M.D., AND ABE A. AARONSON, M.D., CHICAGO, ILL.

Meticorten or prednisone is one of a new series of synthetically prepared crystalline corticosteroids. It differs from the cortisone nucleus by a double bond between C1 and C2.

Reports of its use in rheumatoid arthritis show prompt falls in the urinary 17-ketosteroids and a reduction in the number of circulating eosinophiles. No disturbing side effects, such as fluid retention, weight gain, or sodium increase, were noted. These side effects have limited the usefulness of cortisone, hydrocortisone, and ACTH in such a chronic recurrent disease as bronchial asthma. Therefore, a clinical study of fifty severe asthmatic patients was undertaken in January, 1955, to determine whether Meticorten would alleviate the symptoms of bronchial asthma without the complicating side reactions encountered by the use of cortisone, hydrocortisone, and ACTH. Meticorten was used in doses ranging from 5 mg. to 30 mg. per day. Some patients responded to as little as 5 mg. per day while the severe intractable asthmatic patients required dos-

ages of 30 mg. a day for five to seven days to bring their symptoms under control. These patients all had previously been on cortisone, hydrocortisone, or ACTH.

Clinically, there was no evidence of weight gain, hypertension, or Cushing's syndrome. Patients were all controlled on small doses of Meticorten with a gradual reduction and elimination of the drug. During the period of control of asthmatic symptoms, diagnostic tests are given and continued, specific hyposensitization plus elimination factors are carried out. Charts showing side effects and variation in dosage are presented.

31. BLOOD PROTEIN ABNORMALITIES IN ASTHMATIC CHILDREN

HAROLD S. TUFT, M.D., DENVER, COLO.

In an attempt to discover factors which characterize the intractable asthmatic state in childhood, blood proteins of 115 patients at the Jewish National Home for Asthmatic Children were studied. Sera from these patients were subjected to the following tests: zinc turbidity, zinc flocculation, thymal turbidity, cephalin flocculation, colloidal gold, A/G ratio, and paper electrophoresis. Fifty-two per cent of the sera showed cephalin cholesterol flocculation. A similarly high percentage showed abnormalities of the zinc turbidity, zinc flocculation, and thymal turbidity, but there was definite disturbance of the A/G ratios in most sera. Statistical analysis of the findings of paper electrophoresis demonstrated significant changes over normal values.

The details of this study form the basis of this paper. The pathogenesis of cephaline cholesterol flocculation and colloidal gold changes and the significance of these factors and the electrophoretic pattern changes will also be discussed. Finally, the influence of steroid hormones on these tests will be presented.

32. EYE TESTS WITH INHALANT ALLERGENS—THEIR INDICATIONS AND CLINICAL VALUE

LOUIS TUFT, M.D., L. N. ETTELSON, M.D., AND CHARLES KRUEGER, B.S., PHILADELPHIA, PA.

The present study was made to determine the clinical value of eye tests as additional means of proving clinical importance of positive skin test reactions to inhalant allergens. The study utilized the same dry powdered allergens as for scratch tests, testing important inhalants, pollens, and molds. Sufficient control tests were done to detect inherent nonspecific or irritant qualities of the materials. An effort was made to develop a standardized technique, determining which part of eye was best adapted for instillation, the use of small equal amounts for each test, and whether repeated tests could be done in succession in the same eye to speed up the procedure; also, to ascertain the clinical value of positive tests by checking with history and, whenever possible, by nasal tests, especially with pollens and molds.

Extensive clinical experience with this test thus far indicates that it can be a useful diagnostic adjunct, deserving of more extensive use. Thus, we were able to show that as many as eight to ten tests with inhalant allergens could be done at one sitting, making the procedure more practical; also, that the test was useful in patients with vague clinical histories but positive skin tests, especially to inhalants like house dust, atmospheric molds, and pollens, and to help in deciding which allergens to include in attempting desensitization. It was also useful in patients in whom nasal symptoms prevented per-

formance of nasal tests; also, as a means of comparing various solutions and drops generally utilized for instillation into the conjunctival sac in treatment of allergic conjunctivitis (e.g., epinephrine, cortisone, estivin, etc.). Results of the latter are included in the present report, as well as a citation of specific clinical cases to exemplify instances in which the test was helpful. Possible advantages of this test, as well as the discrepancies between results obtained, the clinical history, and nasal tests, also are pointed out.

33. RAGWEED POLLEN DESENSITIZATION IN HAY FEVER RELATIONSHIP TO SKIN TEST REACTION, DOSAGE, AND CLINICAL RESULTS

LOUIS TUFT, M.D., AND V. MURIEL HECK, M.T., PHILADELPHIA, PA.

The case records of 200 ragweed hay fever patients receiving treatment regularly for periods of one to ten years or longer, both preseasonally and perennially, who had been retested at frequent intervals were reviewed to determine the effect of pollen desensitization upon the alteration in skin test reaction, upon the results of treatment, upon its relationship to dosage and to the length of time treated, and also the effect of different forms of treatment. The results obtained permitted the following conclusions:

1. A decrease in the skin test reaction may follow pollen desensitization, but it is more likely from high pollen dosage and in those treated more than five years, regardless of the method employed. It seldom becomes negative. No reliance should be placed upon such a decrease as an indication for the cessation of treatment.
2. Pollen therapy in hay fever usually will produce good results, but this is more likely in patients whose skin test reactions decrease and in those who can take high doses of pollen extract.
3. Longer treatment brings better clinical results; thus, significantly better results were obtained in those treated five years or more than in those treated for shorter periods.
4. The method of treatment, whether perennial or preseasonal, does not significantly influence either the results obtained or the change in skin test reaction, although the dosage of pollen extract is likely to be higher in the perennial group, probably because of special selection.
5. The results of these investigations help not only in relation to desensitization with pollen extracts, but also with other allergenic extracts employed in allergic conditions other than hay fever.

SCIENTIFIC EXHIBITS

Rehabilitation of the Asthmatic Child. William B. Steen, M.D., Tucson, Arizona.

Aerosolization Techniques in the Treatment of the Asthmatic Child. Joseph B. Miller, M.D., Mobile, Ala.

Usefulness of Prednisone and Prednisolone in Allergic Disorders. Earl B. Brown, M.D., New York, New York.

Mold Survey Committee of the American Academy of Allergy. David Merksamer, M.D., New York, New York.

Pollen Survey and Herbarium Committee of the American Academy of Allergy. Oren C. Durham, North Chicago, Illinois.

A Graphic Representation of the Jewish National Home for Asthmatic Children in the Rehabilitation of the Intractable Asthmatic Child. Harold S. Tuft, M.D., Denver, Colorado.

Seborrheic Dermatitis and Atopic Dermatitis in Infancy and Childhood. Jerome Glaser, M.D., Rochester, New York.

Adrenal Steroid Therapy in Allergic Diseases. Emanuel Schwartz, M.D., Brooklyn, New York.

Announcements

Michigan Allergy Society

The following officers have been elected by the Michigan Allergy Society for the current year:

President, Sidney Friedlaender, Detroit, Mich.
Vice-President, Kenneth Mathews, Ann Arbor, Mich.
Secretary-Treasurer, E. Oskar Schreiber, Flint, Mich.

Pennsylvania Allergy Association

At a meeting of the Pennsylvania Allergy Association held at Hotel William Penn in Pittsburgh on Sept. 21, 1955, the following officers were elected for the year 1956.

President, Lester L. Bartlett, Pittsburgh, Pa.
President-Elect, James Flood, Sayre, Pa.
Secretary-Treasurer, Ralph Mulligan, Reading, Pa.
Board of Regents, Charles Cleland, Kane, Pa.
Jean Crump, Philadelphia, Pa.

The spring meeting of the Association will be held at Galen Hall, Wernersville, Pennsylvania, May 17 to 20, 1956.

Residency in Allergy

A residency in allergy at the Ohio State University Health Center is available July 1, 1956.

Facilities include in- and outpatients, adults and children, clinical and private services. There are excellent opportunities for experience in related subspecialties, including dermatology, pulmonary diseases, and cardiology.

For particulars, write John H. Mitchell, M.D., 1625 Perry St., Columbus 1, Ohio.

Book Reviews

Allergy Cooking. A Guide With Menus and Recipes. By Marion L. Conrad, New York, Thomas Y. Crowell Company, 1955, 380 pages. Price, $5.00.

Most diets prescribed for allergic patients employ a negative approach; the use of a number of the most important staple foods is forbidden, and the patient is left to shift for himself. Many physicians, who often tell patients what foods to avoid, would be hard put to tell them what foods they can eat. This book, written by an experienced dietician who has herself followed restricted diets for years and prepared them for members of her family, makes the familiar and usually dull milk-, egg-, and cereal-free diet an excuse for enjoying a tremendous variety of appetizing new dishes. For breakfast, which is always a problem on a diet of this type, sixteen attractive menus are offered, most of which would be a welcome change for a person not affected by food allergy.

The problems of eating out, in restaurants and private homes, at parties and celebrations, and even at picnics and on camping trips, are discussed, and practical advice is offered.

While it includes some bits of medical information on the scope of food allergies and the importance of false tolerances for foods which may not be acceptable to all physicians interested in this field, the book is primarily a cookbook and list of menus, and as such should be very useful.

Systemic Lupus Erythematosus: Review of the Literature and Clinical Analysis of 138 Cases. By A. M. Harvey, M.D., L. E. Shulman, M.D., P. A. Tumulty, M.D., E. L. Conley, M.D., and E. H. Schoenrich, M.D., Baltimore, Williams & Wilkins Company, 1955, 147 pages. Price, $3.00.

This little book is a reprint from *Medicine* **33**: No. 4, December, 1954, but its authoritative coverage of the subject well warrants publication in book form. All aspects of the disease are reviewed and data are supplied from the rather large group of cases carefully studied and treated at the Johns Hopkins Hospital. The prolonged course of the disease with varying symptoms and remissions is well brought out. The importance and reliability of the L. E. cell as an early diagnostic test are stressed, no false positives being found in over 800 tests done, and positive results being obtained in seventy-nine of ninety-six patients with lupus examined. Results of treatment of sixty-two patients with cortisone or corticotropin are analyzed in detail. The dosage used was generally conservative and treatment was intermittent in most cases.

The whole treatment is factual, and the physician interested in allergy may regret the reluctance of the authors to speculate on the role of hypersensitivity in the etiology of lupus and on the interpretation of the cases resembling lupus which develop after treatment with Apresoline.